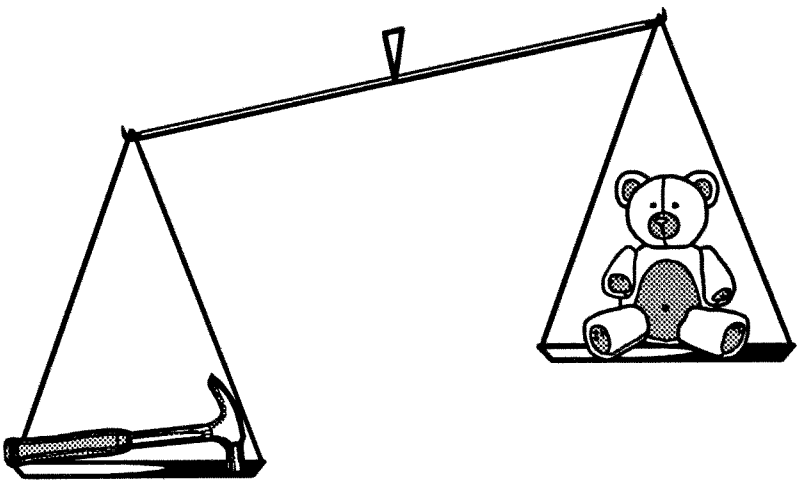
Near Infrared Spectroscopy: Toy or Tool?

An investigation on the clinical applicability of Near Infrared Spectroscopy



Willy N.J.M. Colier

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Een wetenschappelijke proeve op het gebied van de
Medische Wetenschappen

Proefschrift

Ter verkrijging van de graad van doctor aan de
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door

Wilhelmus Nicolaas Josephus Maria Colier
geboren 25 juni 1960

Contents

Chapter 1

Introduction

Willy N.J.M. Colier

A short history of oximetry

During the second half of the 19th century it was discovered that blood cells contain a colouring substance whose spectrum is influenced by various blood gases [Hoppe, 1862]. Stokes discovered that this coloured substance was the oxygen carrier of the blood. Felix Hoppe-Seyler, a German chemist, named the substance haemoglobin. He found that when shaking a solution of haemoglobin with air this resulted in a spectral change. He called the component responsible for this change "oxyhaemoglobin". In 1876 Karl von Vierordt published the first study in which spectroscopy was used to study haemoglobin in tissue. He found a transition from oxyhaemoglobin to deoxyhaemoglobin in the tissue of the finger after the arm was fully occluded, a technique still practised today to study oxygenation in muscles (refer to Figure 1.2 at page 16).

At the begining of the 20th century oximetry developed rapidly in Germany with scientists like Nicolai [1932], Kramer [1934], Matthes [1942] and Gross. Last two scientists mentioned were the first to use a wavelength region in the near infrared where absorption was independent of the oxygenation status of blood and which could therefore compensate for changes in blood volume, light scattering and tissue thickness [Matthes *et al.* 1939]. Their instrumentation however was still very bulky and not easy to use.

The first portable instrument measuring haemoglobin saturation in tissue accurately and automatically was built and described by Millikan [1942]. He named the instrument an "oximeter". The sensor weighed 30 grams, used two wavelengths and could be slipped over the subject's ear. To obtain arterial saturation the ear was moderately heated, a method also used in later versions of oximeters. Millikan's instrument had an accuracy of 3 to 5% at the higher end of the scale (~98% saturation) and an accuracy of 8% at the lower end of the scale (~50% saturation).

The oximeter of Millikan was improved by Wood and Geraci [1949]. Further developments on reflection oximetry were done by Brinkman and Zijlstra. They developed the "Cyclops", a device measuring oxygen saturation on the forehead of a subject [Brinkman *et al.* 1950]. In the following years, oximetry was used more and more in clinical situations. The title of Zijlstra's thesis [1951]: "Fundamentals and applications of clinical oximetry" indicated that they were far ahead of their time.

In the seventies two important findings contributed to the development and clinical applicability of oximetry. The first was by Aoyagi, who found that the variations in arterial blood volume could be used to obtain a signal dependent only on arterial blood changes. With this knowledge the arterial oxygen saturation of the blood could be measured [Aoyagi *et al.* 1974, Nakajima *et al.* 1975]. This technique

was called pulse oximetry and has been developed into a reliable and widely used method [Kelleher 1989].

The second finding was by Jöbsis [1977] and was the beginning of near infrared spectroscopy (NIRS).

Near Infrared Spectroscopy

In his article in Science [1977] Jöbsis reported that biological tissues have a relatively good transparency for light in the near infrared region (700-1300 nm). Therefore it is possible to transmit sufficient photons through organs for *in situ* monitoring. In this region haemoglobin, which can be divided into its main components oxyhaemoglobin (O_2Hb) and deoxyhaemoglobin (HHb), shows oxygen dependent absorption. The main attention by Jöbsis and his co-workers however was given to the redox state of cytochrome a,a_3 (Cyt.ox), the terminal enzyme of the mitochondrial respiratory electron transport chain. Approximately 90% of the intracellular oxygen consumption is catalyzed by this enzyme. It has been shown that the absence of oxygen results in a complete reduction of Cyt.ox. This reduction can be monitored by measuring the change in absorption band of Cyt.ox in the near infrared region (770-880 nm) [Chance 1966]. Information gained from the near infrared spectrum can therefore give insight into the oxygen availability at the intracellular level. Combined with the information from the haemoglobin signal it is now possible to monitor both circulatory oxygen supply and intracellular oxygen consumption of the tissue.

Table 1.1 The history of Near Infrared Spectroscopy (NIRS) in milestones

Year	*Authors*	*Subject*
1977	Jobsis	First publication on NIRS
1982	Giannini *et al*	Cyt ox monitoring after perfluorcarcon exchange
1985	Brazy *et al*	Neonatal brain monitoring
1986	Ferrari *et al*	Adult brain monitoring
1986	Wyatt *et al*	Quantitation of cerebral oxygenation in newborn
1988	Delpy *et al.*	Quantitation of optical pathlength
1988	Edwards *et al*	Quantitation of cerebral blood flow
1990	Wyatt *et al*	Quantitation of cerebral blood volume
1991	de Blasi *et al*	Quantitation of oxygen consumption in muscle

In the years following the first publication of Jöbsis many studies were carried out focusing on the assessment of Cyt.ox within the brains of animals or humans, especially neonates [Jöbsis 1979, Giannini *et al.* 1982, Keizer *et al.* 1985, Proctor *et al.* 1985, Brazy *et al.* 1985, Brazy and Lewis 1986, Jöbsis-VanderVliet *et al.* 1987, Brazy 1988, Jöbsis-VanderVliet *et al.* 1988]. Due to technical improvements NIRS became a

more widespread method at the end of the eighties and into the nineties. Most clinical studies still are in the neonatal field [Brazy 1991, Edwards *et al.* 1991, Livera *et al.* 1991, Skov *et al.* 1991, Wickramasinghe *et al.* 1992, Skov *et al.* 1992, McCormick *et al.* 1993, Bucher *et al.* 1993], some of them performed by the Nijmegen group [Liem *et al.* 1992, 1994, 1995].

Principles of NIRS

The technique of NIRS relies on the Lambert-Beer law [Beer 1851], given by:

$$OD_\lambda = Log\left(\frac{I_o}{I}\right) = \varepsilon_\lambda \cdot c \cdot L$$

where OD_λ is a dimensionless factor known as the optical density of the medium, I_0 is the incident radiation, I the transmitted radiation, ε_λ the extinction coefficient of the chromophore ($mM^{-1} \cdot cm^{-1}$), c is the concentration (mM^{-1}) of the chromophore, L the distance (cm) between light entry and light exit point and λ the wavelength (nm) used. In this case the Lambert-Beer law is given for a system with a single component.

The Lambert-Beer law was intended for use in a clear, non-scattering medium. When the law is applied to a scattering medium, e.g. biological tissue, a dimensionless pathlength correction factor B must be incorporated. This factor, sometimes called "Differential Pathlength Factor (DPF)", accounts for the increase in optical pathlength due to scattering in the tissue. The modified Lambert-Beer law [Delpy *et al.* 1988] is given by:

$$OD_\lambda = \varepsilon_\lambda \cdot c \cdot L \cdot B + OD_{R,\lambda}$$

where $OD_{R,}\lambda$ represents the oxygen indepedent light losses due to scattering in the tissue. Assuming $OD_{R,}\lambda$ is constant during a measurement we can convert an optical density change into a concentration change:

$$\Delta c = \frac{\Delta OD_\lambda}{\varepsilon_\lambda \cdot L \cdot B}$$

This equation is valid for a medium with one chromophore. In the case of more chromophores we need to measure at at least as many wavelengths as there are chromophores present. This results in a set of linear equations. The solution of this set leads to the algorithm used in most NIRS systems (see also Chapter 2).

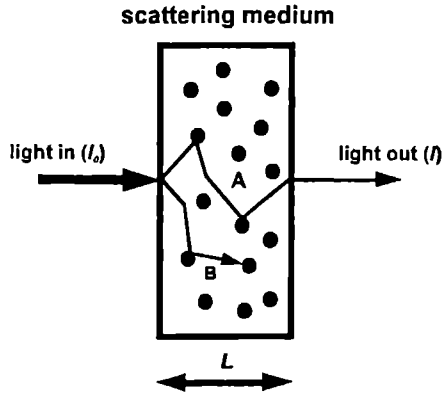

Figure 1.1 A scattering medium with an incident and transmitted light ray. The chromophore is symbolized by black dots. Light ray A is scattered, and therefore travels a distance which equals the pathlength correction factor times the physical pathlength *L*. Light ray B is absorbed completely after being scattered.

In biological tissue there are at least three oxygenation dependent chromophores present: O_2Hb, HHb and Cyt.ox. The sum of O_2Hb and HHb is a measure for the total blood volume (tHb) in the tissue. If muscle tissue is investigated there are two more chromophores present: oxy- and deoxymyoglobin (O_2Mb and HMb).

Spectral extinction coefficients of the chromophores

To define the algorithm the spectral extinction coefficients of the various chromophores are needed. Those first to explore extensively the spectra of haemoglobin were Hüfner [1900] and Horecker [1943]. More recent is an investigation of van Assendelft [1970] who studied haemoglobin and its derivatives. Due to the introduction of NIRS and pulse oximetry several recent studies have focused on the spectra of haemoglobin and cytochrome oxidase with special attention to the near infrared spectral region [Rea *et al.* 1985, Harris *et al.* 1988, Wray *et al.* 1988, Zijlstra *et al.* 1991, Crowe 1994]. Harris and Zijlstra determined absorption spectra of both fetal and adult haemoglobin. This is of importance as pulse oximetry and NIRS are methods used in fetal and neonatal applications.

Small differences in absorption spectra might influence the quantitation of the data. Studies of Wickramasinghe *et al.* [1993] and Colier *et al.* [1992] showed that the transition from fetal to adult haemoglobin had no significant influence on the quantitation of the haemoglobin data. Care however has to be taken with the quantitation of cytochrome oxidase, e.g., during blood tranfusion or extra corporeal membrane oxygenation (ECMO), interventions where a transition from fetal to

adult haemoglobin takes place. A small change in the algorithm can then leed to unacceptable high errors in the Cyt.ox signal. The change in Cyt.ox is highly associated with changes in haemoglobin [Skov *et al.* 1994]. Therefore no attention is paid to Cyt.ox in the *in vivo* experiments of this thesis.

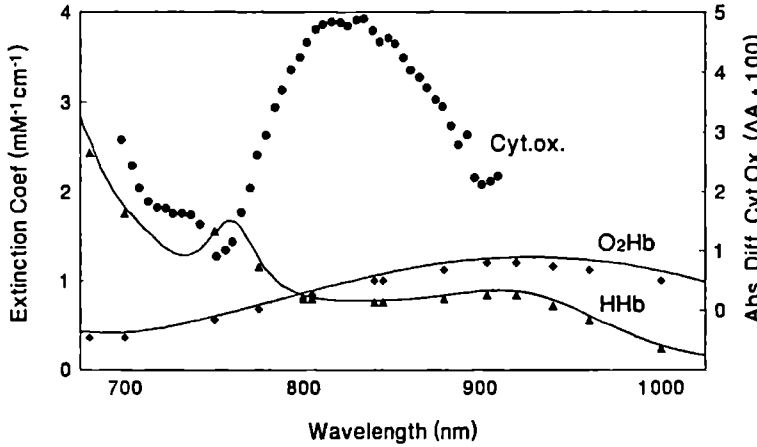

Figure 1.2 Extinction coefficients of adult oxy- and deoxyhaemoglobin (O_2Hb and HHb respectively), determined by Wray [1988] (solid lines) and Zijlstra [1991] (▲ and ◆ symbols), and cytochrome oxidase (Cyt.ox, ● symbol), also from Wray [1988].

To distinguish between haemoglobin and myoglobin in muscle tissue the spectra need to be sufficiently different. Unfortunately this is not the case in the visible part of the spectrum [Theorell *et al.* 1955, Yamazaki *et al.* 1964, Samejima *et al.* 1964, Hardman *et al.* 1965]. About the near infrared part of the spectrum only one publication is known [Thorniley *et al.* 1990], showing a different spectrum of O_2Mb compared to O_2Hb. This finding however has not yet been confirmed.

Up until now there exists no consensus about which of the data sets for haemoglobin and cytochrome oxidase come closest to reality. It can therefore be concluded that the existence of several spectral data sets results in a non-uniform algorithm for NIRS.

The pathlength correction factor

For the quantitation of the absorption changes not only the physical pathlength L, or inter-optode distance, is needed, but also the DPF. The DPF can be assessed via different ways. The most common way is by "time-of-flight" measurements [Delpy *et al.* 1988, Wyatt *et al.* 1990a, Ferrari *et al.* 1992, van der Zee *et al.* 1992]. An ultrashort

laser pulse is fired into the tissue. The pulse is detected by an ultrafast camera. The time of flight t can than be converted into a travelled distance d using the formula:

$$d = \frac{c \cdot t}{n}$$

where c is the velocity of light and n the refractive index of the tissue. Division of d by L gives the DPF.

Table 1.2 Values of the Differential Pathlength Factor (DPF) for various types of tissue determined by either "time-of-flight" (TRS) or "frequency-resolved" (FRS) measurements. All DPF values are given as mean ± S.D.

Author	*Method*	*Organ/Limb*	*Male/Female*	*Wavelength (nm)*	*DPF*
Wyatt, 1990a	TRS	Baby Head (✞)	?	783	4.39 ± 0.28
van der Zee, 1991	TRS	Baby Head (✞)	M/F	783	3.85 ± 0.57
Benaron, 1990	FRS	Infant Head	M/F	754	3.78 ± 0.31
Benaron, 1990	FRS	Infant Head	M/F	816	3.71 ± 0.30
Duncan, 1995	FRS	Infant Head	M/F	807	4.99 ± 0.45
van der Zee, 1991	TRS	Adult Head	M/F	761	5.93 ± 0.42
Essenpreis, 1993	TRS	Adult Head	M/F	800	6.32 ± 0.46
Duncan, 1995	FRS	Adult Head	M/F	807	6.26 ± 0.88
Essenpreis, 1993	TRS	Adult Calf	M	800	5.84 ± 0.65
Essenpreis, 1993	TRS	Adult Calf	F	800	5.63 ± 0.62
van der Zee, 1991	TRS	Adult Calf	M	761	3.98 ± 0.46
van der Zee, 1991	TRS	Adult Calf	F	761	5.14 ± 0.43
Duncan, 1995	FRS	Adult Calf	M	807	4.94 ± 0.67
Duncan, 1995	FRS	Adult Calf	F	807	6.09 ± 0.93
Duncan, 1995	FRS	Adult Forearm	M	807	3.74 ± 0.57
Duncan, 1995	FRS	Adult Forearm	F	807	4.57 ± 0.74
van der Zee, 1991	TRS	Adult Forearm	M/F	761	3.59 ± 0.32
Ferrari, 1992	TRS	Adult Forearm	M/F	800	4.3 ± 0.2
Essenpreis, 1993	TRS	Adult Forearm	M/F	800	4.48 ± 0.41

✞: post mortem sample

Although the technique of "time-of-flight" measurements gives good results, the equipment needed for it is expensive and bulky and therefore only available to a few research centres.

Another way of assessing the DPF is by making use of "frequency resolved" systems [Benaron *et al.* 1990, Sevick *et al.* 1991, Duncan *et al.* 1995]. In this technique the incident light is intensity modulated at a frequency of 200-300 MHz. A phase sensitive detector measures the envelope phase shift between the incident and transmitted light. From the phase shift the mean pathlength can be obtained. A good

set of pathlength data obtained with this technique for brain and muscle tissue has recently been published by Duncan [1995]. At this moment however the technique is not yet available for clinical use, but in the future it will most likely be possible to obtain real-time pathlength information.

Occlusion methods in NIRS

By applying an arterial or a venous occlusion to a limb it becomes possible to assess various haemodynamic variables, like limb blood flow or oxygen consumption of muscle tissue during rest or during exercise.

Venous occlusion technique

When a venous occlusion is applied to the upper arm or leg by inflating a blood pressure cuff to a pressure of approximately 50 mmHg this results in (arterial) inflow of blood but no outflow. The observed rise in blood volume equals the blood flow into the limb and can be measured with NIRS by monitoring the rise of the tHb signal after the occlusion. This method has been validated with strain gauge plethysmography by de Blasi *et al.* [1994]. Figure 1.3 gives an example of a NIRS tracing during venous occlusion of the arm.

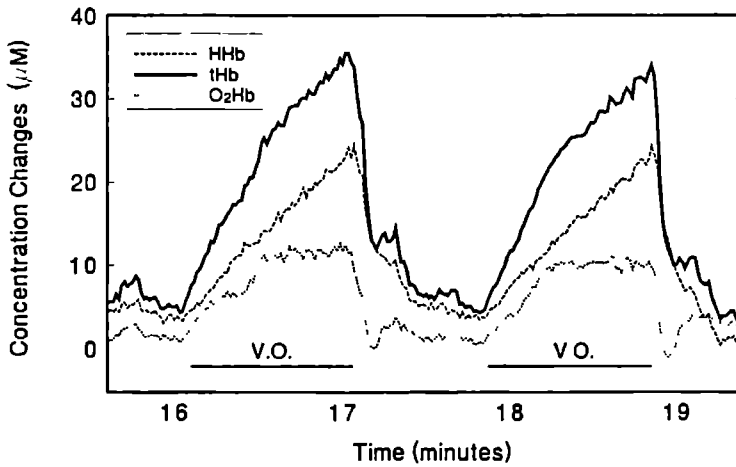

Figure 1.3 An example of a NIRS tracing. In this case two consecutive venous occlusions (V.O.) were applied to the upper arm. The NIRS optrodes were attached to the brachio-radialis muscle of the forearm. During V.O. there is arterial inflow, but no venous outflow. This results in an increase of both oxy- and deoxyhaemoglobin. From the rise in the tHb signal the blood flow into the limb can be calculated.

Arterial occlusion technique

The blood flow into a limb can be completely stopped by inflating a blood pressure cuff to a pressure of more then 250 mmHg. It is then possible to calculate the oxygen consumption ($\dot{V}O_2$) in rest [Cheatle *et al.* 1991, de Blasi *et al.* 1993] or during (isometric) exercise [Colier *et al.* 1995] from the gradient of the subsequent decrease of the O_2Hb signal. An example of NIRS tracings during an arterial occlusion is given in Figure 2 of Chapter 6, where this technique is used to measure $\dot{V}O_2$ of the forearm as a function of a percentage of the maximum voluntary isometric contraction.

After the pressure of the cuff is released the tissue will show a hyperaemic reaction. The recovery time of the resaturation observed with NIRS can be used as measure for, e.g., the vascularisation of the leg in patients with peripheral vascular disease [McCully *et al.* 1994, Komiyama *et al.* 1994].

Quantitation of absolute tissue blood volume

By combining the NIRS data with arterial saturation (SaO_2), measured for example by pulse oximetry, it is possible to quantitate the absolute value of blood volume of the examined tissue. This method was first described by Wyatt *et al.* [1990b] for the quantitation of cerebral blood volume.

The effect of a small, gradual and transient change in SaO_2 on the O_2Hb concentration is monitored. A decrease in SaO_2 of approximately 10%, induced by lowering the inspired oxygen concentration, is sufficient to calculate the blood volume. Provided blood flow, volume and oxygen consumption remain constant during the procedure, the tissue blood volume (TBV) in ml/100 g is given by:

$$TBV = \frac{\Delta(O_2Hb - HHb)}{2 \cdot R \cdot \Delta SaO_2} \cdot c_{Hb} \cdot \rho_T \cdot k$$

where c_{Hb} (mM) is the haemoglobin concentration of whole blood, ρ_T (g/cm^3) the specific density of the tissue, k a constant reflecting metric conversions and R is, in case of cerebral tissue, the large-to-small vessel haematocrit ratio with a value of 0.69 [Lammertsma *et al.* 1984]. The difference between the O_2Hb and HHb concentration is taken to obtain a better signal-to-noise ratio. This difference is also called the oxygenation index (OI).

Using this method an absolute change in arterial saturation is compared to a relative change in concentration of O_2Hb, which can than be quantified. This method is applied in chapters 4 and 5 to quantitate testicular blood volume.

Quantitation of absolute blood flow

The principle of measuring organ blood flow with NIRS is based on the Fick principle which states that the accumulation of a tracer in an organ equals the difference between the inflow (arterial concentration x flow) and outflow (venous concentration x flow). If we measure within the transit time of the tracer through the organ the venous concentration will be zero. In NIRS the tracer used is a bolus of O_2Hb, which can be induced by suddenly increasing the inspired oxygen concentration. The concentration of the bolus can be measured by attaching a pulse oximetry probe onto the organ. The increase of O_2Hb as measured by NIRS represents the accumulation of the bolus into the organ. The blood flow (*BF*, in $ml \cdot 100g^{-1} \cdot min^{-1}$) through the organ is then given by the change of O_2Hb devided by the product of the arterial haemoglobin concentration (*ctHb*, in $g \cdot ml^{-1}$) times the integral of change in arterial saturation (SaO_2, in %) :

$$BF = \frac{K \cdot \Delta(O_2Hb)}{ctHb \cdot 10^{-2} \cdot \int_0^t \Delta(SaO_2)dt}$$

K is constant representing the molecular weight of haemoglobin, the tissue density and a metric conversion factor. This methodology has first been described by Edwards *et al.* [1988] for the determination of cerebral blood flow in newborn and afterwards by others who made a comparison with the ^{133}Xe clearance method [Skov *et al.* 1991, Bucher *et al.* 1993], finding an acceptable correlation between the two methods in newborn. Elwell *et al.* [1992, 1993] have used it to determine the cerebral blood flow in adults.

Some of the disadvantages of the methodology are firstly that a certain degree of hypoxia with subsequent hyperoxia is needed to induce the O_2Hb bolus. If this intervention is inert is not known. Furthermore an adequate lung function is necessary. Secondly a reliable beat-to-beat pulse oximeter is needed for the measurements, which is generally a problem.

Aim of this study

Since the first publication of Jöbsis [1977] on NIRS the method has mainly been used to monitor the cerebral circulation and oxygenation of neonates and newborn. Only few studies showed that NIRS could be applied to other organs and tissues. Monitoring of skeletal muscle oxygenation was one of these areas [Piantadosi *et al.*

1986, Hampson *et al.* 1987]. Another, however scarcely explored area in the mid-eighties was the adult cerebral circulation [Ferrari *et al.* 1985, Glaister 1988]. An interesting study was published by Ferrari [1987], showing that NIRS could be a valuable tool in the evaluation and the diagnosis of carotid stenotic disease.

All of the studies mentioned were descriptive in nature. At the end of the eighties methods were developed to quantitate the relative NIRS signals or correlate them with other physiological measurements [Wyatt *et al.* 1986, Cope *et al.* 1989, Greisen *et al.* 1989] to gain more information. The quantitation of the NIRS signals made it possible to compare different studies. Some authors nevertheless still held on to arbitrary units, like Jöbsis-vanderVliet using "Vanders".

The aim of this study was to validate and examine some of these methods and to explore new fields of research and clinical applications of NIRS. Chapter 2 gives an evaluation of the algorithm used in NIRS. Chapter 3 compares a "gold standard" NIRS instrument with an instrument with assumed quantifying capability during hypo- and hypercapnic interventions in adults. Chapters 4 and 5 describe how NIRS can be used as a diagnostic tool in patients with cryptorchidism (undescended testis). In chapter 6 NIRS is used to quantify oxygen consumption in human muscle during isometric graded excercise. Chapter 7 describes how the cerebral circulation behaves under influence of orthostatic stress. The thesis ends with an Epilogue and Summary.

Evaluation of the algorithm used in Near Infrared Spectrophotometry

Willy N.J.M. Colier, Biny E.M. Ringnalda, Jos A.M. Evers and Berend Oeseburg

(Adv Exp Med Biol 1992; 305-311)

Introduction

The major causes of death and disability in a neonatal intensive care unit (NICU) are cerebral ischemia and intraventricular haemorrhage. The monitoring of cerebral oxygenation and haemodynamics is of utmost importance for the care of the preterm infant. A small, inexpensive and continuous measuring bedside instrument is a substantial extension of the NICU. The technique of near infrared spectrophotometry (NIRS) might provide in this need.

The technique is based on the relative high transparency of skin and skull for light in the near infrared region. This allows photon transmission through the brain. For the brain, it is assumed that changes in light absorption are caused by oxy- and deoxyhaemoglobin (O_2Hb and HHb) and by the cytochrome a,a_3 oxidation level.

To monitor this three component system, a minimum number of three wavelengths is needed. The optical density *OD* can be written as:

$$\begin{pmatrix} OD(\lambda_1) \\ OD(\lambda_2) \\ OD(\lambda_3) \end{pmatrix} = \begin{pmatrix} \alpha_{O_2Hb}(\lambda_1) & \alpha_{HHb}(\lambda_1) & \alpha_{Cyt}(\lambda_1) \\ \alpha_{O_2Hb}(\lambda_2) & \alpha_{HHb}(\lambda_2) & \alpha_{Cyt}(\lambda_2) \\ \alpha_{O_2Hb}(\lambda_3) & \alpha_{HHb}(\lambda_3) & \alpha_{Cyt}(\lambda_3) \end{pmatrix} \cdot \begin{pmatrix} c_{O_2Hb} \\ c_{HHb} \\ c_{Cyt} \end{pmatrix} \cdot L + \begin{pmatrix} OD_R(\lambda_1) \\ OD_R(\lambda_2) \\ OD_R(\lambda_3) \end{pmatrix}$$

where *L* denotes the optical pathlength through the medium (cm), α the absorption coefficient ($mM^{-1}cm^{-1}$) and *c* the concentration (mM) of the absorbing components. OD_R presents the oxygen independent absorption losses caused by skin, skull and other tissue. The equation is based on Lambert-Beer's law.

If OD_R is assumed to remain constant during measurements, and by looking at changes of the absorption signal, OD_R will, by subtraction, cancel out. Solving the set of linear equations will result in the algorithm for use in NIRS.

$$\Delta\vec{c}_{comp} = \vec{\alpha}^{-1} \cdot \Delta\vec{O}D(\lambda_n) \; / \; L$$

$\vec{\alpha}^{-1}$ represents the inverted matrix of the absorption coefficients for the different components at the wavelengths used in the system. Several authors have published the inverse absorption values used in their work [Cope *et al.* 1987, Reynolds *et al.* 1988, Livera *et al.* 1991]. Extensive research into the absorption coefficients of haemoglobin and/or cytochrome has been done by Wray *et al.* [1988], Rea *et al.* [1985] and Zijlstra *et al.* [1991]. However, the values of the inverse absorption matrix used by some research groups show differences, although the same mathematics is used. In our opinion this makes a basic evaluation of the coefficients used necessary. In this study the coefficients used by the group of Rolfe, University of Keele, Great Britain and by the group of Delpy, University College London, Great Britain were

investigated. Also the algorithm of Radiometer Medical A/S, Copenhagen, supplied by their near infrared spectrophotometer, was investigated.

The studies were performed in an *in vitro* system in which the oxygen saturation of the haemoglobin could be altered. Upon same sets of absorption data, obtained in the *in vitro* system, calculations were performed to obtain the oxy- and deoxyhaemoglobin concentration and the saturation, which can be calculated by dividing the oxyhaemoglobin concentration by the total haemoglobin (tHb) concentration, being the sum of oxy- and deoxyhaemoglobin. Also the cytochrome oxidation level was calculated. However, as in both the haemoglobin solutions used in this study no cytochrome is present we should detect a constant (zero) level. For quantitation of the changes in the haemoglobin concentration and the cytochrome oxidation level the optical pathlength of the photons in the medium must be known [Delpy *et al.* 1988, Wyatt *et al.* 1990a].

Materials and methods

Haemoglobin solutions

Two types of solutions were used. The first was a, ready to use, human erythrocyte suspension, without leukocytes, obtained from the Red Cross Blood Bank.

The second type was a human stroma-free haemoglobin solution. This solution was obtained by shaking one part of an erythrocyte suspension with two parts of distilled water, then storing it in a refrigerator. After one hour the solution is frozen on a mixture of dry ice and acetone. After thawing, the mixture is spun and filtered to below 0.45μm particle size to give a clear, stroma-free haemoglobin solution.

Experimental setup

The solution under investigation was pumped through a 5mm cuvette and a membrane oxygenator. Saturation was changed by varying the composition of the gas supply, a humidified oxygen-nitrogen-carbon dioxide mixture. The haemoglobin concentration was varied by dilution with saline. Concentrations of methaemoglobin, carboxyhaemoglobin, oxy- and deoxyhaemoglobin were measured using an oximeter (IL 482, Instrumentation Laboratory, Italy). The absorption was measured with a near infrared spectrophotometer (Radiometer Medical A/S, Denmark) at wavelengths of 775, 805, 845 and 904nm. Figure 2.1 gives a schematic view of the setup.

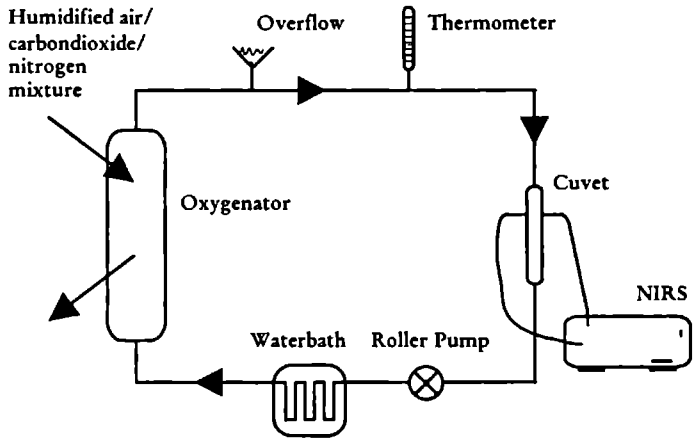

Figure 2.1 Experimental setup for the *in vitro* evaluation of the NIRS coefficients.

Experiments

During an experiment the oxygen saturation of the solution was varied from about 3% to 98%. For faster oxygenation the erythrocyte suspension was kept at a temperature of 28°C. The stroma-free haemoglobin solution was kept at a temperature of 10°C, to avoid formation of methaemoglobin. The measured absorption data were corrected for losses due to divergence and reflection, using a blank sample.

Results and discussion

At first the algorithm was checked for its stability by applying a gradually changing saturation to the system. In Figures 2.2a and 2.2b it can be observed that the total haemoglobin concentration, especially in the case of the stroma-free solution, is not the totally flat curve it should be. The Rolfe and the Delpy coefficients show similar behaviour in the non-scattering case, which also holds for the cytochrome oxidation level. In the case of the Rolfe coefficients we can observe the expected constant zero cytochrome oxidation level. Almost the same result is obtained with the Delpy coefficients. In the case of the Radiometer coefficients an unreliable cytochrome oxidation level is observed.

The differences between the erythrocyte solution and the stroma-free solution are most probably caused by scattering effects. As long as these effects are limited to an offset in the signal without influencing the linearity this has no influence on the final results, as in NIRS only *changes* of the signal are calculated.

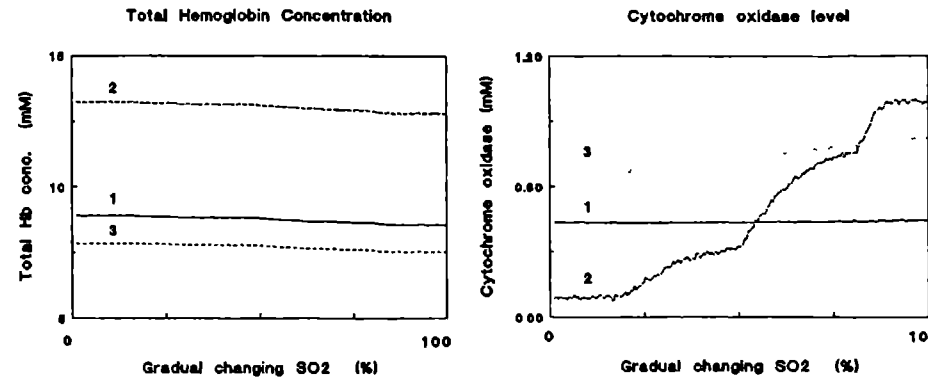

Figure 2.2a The effect of a gradual changing saturation (SO_2) on the total haemoglobin concentration and the cytochrome oxidation level for the scattering erythrocyte suspension (1: Rolfe, 2: Radiometer, 3: Delpy).

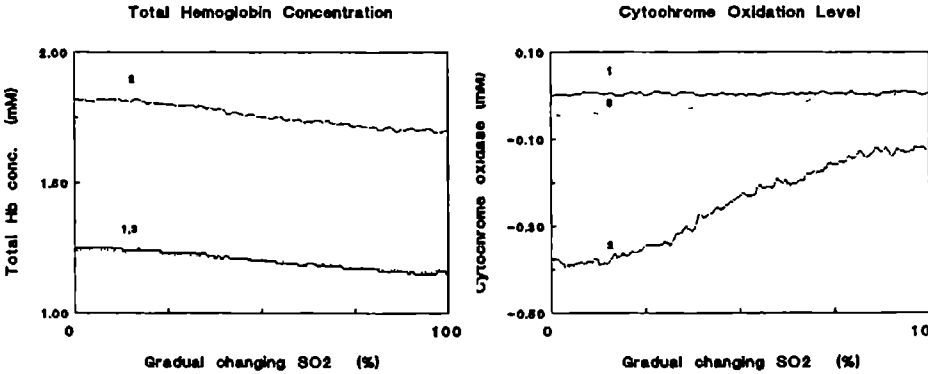

Figure 2.2b The same experiment as in Figure 2.2a, but now a non-scattering stroma-free haemoglobin solution is used (1: Rolfe, 2: Radiometer, 3: Delpy).

When the erythrocyte suspension and the stroma-free haemoglobin solution are diluted, to an extent of more than 50%, we get a linear relation between measured and calculated haemoglobin saturation (Figure 2.3). In a clinical situation this means that changes in (cerebral) blood volume can well be detected. Although having a linear relationship, the concentration values do not match. This is partly due to the scattering effect as their is no correction for the differences between optical and physical pathlength. Another disturbing effect are the system's optical losses, which can not be completely corrected.

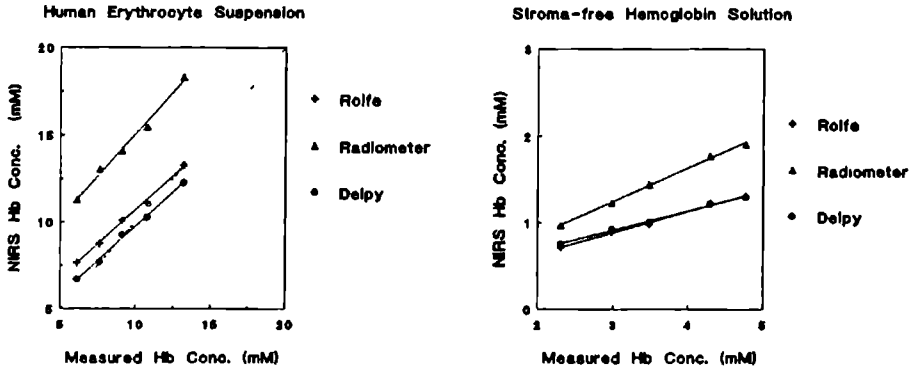

Figure 2.3 The calculated haemoglobin concentration (NIRS) as a function of the measured haemoglobin concentration (oximeter) during dilution

A linear relation was found between the calculated and the measured saturation for the erythrocyte suspension and for the stroma-free haemoglobin solution (Figures 2.4a and 2 4b). Dilution of either of these solutions has no effect on the calculated saturation.

Conclusions

Only minor differences can be observed between the Rolfe and Delpy coefficients. Both are suitable algorithm for use in NIRS with almost identical behaviour, this in contrast with the Radiometer coefficients. In scattering media, incorporation of an optical pathlength factor into the algorithm, eventually analysed per wavelength, is essential for a good result. The cytochrome oxidation level is difficult to derive and needs further investigation.

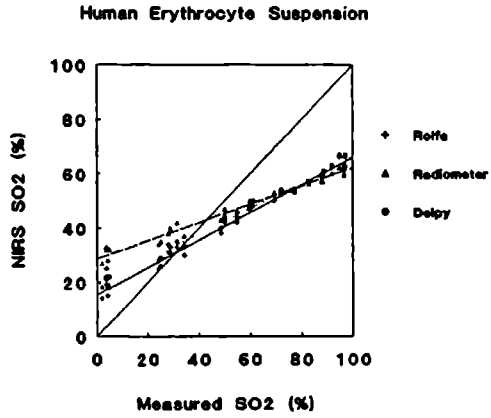

Figure 2.4a The oxygen saturation calculated from the NIRS signals as a function of the measured saturation (oximeter) for the erythrocyte suspension.

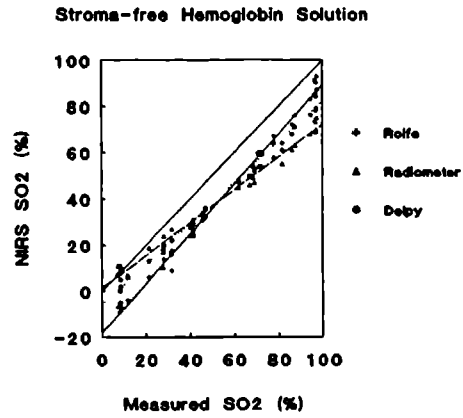

Figure 2.4b The oxygen saturation calculated from the NIRS signals as a function of the measured saturation (oximeter) for the stroma-free haemoglobin solution.

A comparative study of two Near Infrared Spectrophotometers for the assessment of cerebral haemodynamics

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(Acta Anaesthesiol Scand 1995; *in press*)

Summary

Conventional near infrared spectroscopy (NIRS), introduced by Jöbsis in 1977, can be considered as a reliable trend monitor for cerebral oxygenation. Quantitation however is complex and cumbersome. Recently a relatively simple system for cerebral oximetry (INVOS 3100, Somanetics Corporation, USA) was developed, measuring the regional oxygen saturation (rSO_2) in the capillary bed of the cerebrum, presented as a numerical figure for easy interpretation. In this study a comparison was made between a conventional NIRS instrument and the new INVOS instrument, in order to obtain information about sensitivity and usefulness of the INVOS system. Changes in cerebral haemodynamics were induced by a moderate decrease of the arterial oxygen saturation (SaO_2) and by varying the arterial carbon dioxide level ($PaCO_2$). This will result in a higher (hypercapnia) or lower (hypocapnia) cerebral blood flow and subsequent change of both NIRS signals and INVOS signal. Healthy volunteers were used for this study. It was found that the steady state value for rSO_2 was 70 ± 6% (mean ± S.D.). During the lowering of arterial saturation a poor correlation was found between rSO_2 and SaO_2 ($r=0.47$). Increased cerebral blood flow induced by hypercapnia was detected by both conventional NIRS and the INVOS. Decreased cerebral blood flow induced by hypocapnia could only be detected by conventional NIRS. It was concluded that due to the variation in displayed rSO_2 and the high amount of averaging in the algorithm the INVOS instrument does not yet provide more information than conventional NIRS. The displayed value of rSO_2 can even be misleading.

Introduction

Since the introduction of Near Infrared Spectroscopy (NIRS) by Jöbsis in 1977 the technique has mainly been used in a limited number of paediatric wards [Brazy *et al.* 1986, Livera *et al.* 1991, Liem *et al.* 1995]. Most important reason for this is the qualitative nature of the variables obtained directly with this technique. Indirectly it is possible to obtain quantitated variables of the cerebral haemodynamics [Edwards *et al.* 1988, Wyatt *et al.* 1990b]. These variables however can only be obtained via a complex and cumbersome data analysis. Recently a relatively simple system for cerebral oximetry (INVOS 3100, Somanetics Corporation, USA) was developed for clinical investigational use. This system measures the regional oxygen saturation in the capillary bed of the cerebrum, presented as a numerical figure for easy interpretation.

Since there is no 'gold standard' for cerebral oximetry absolute validation of such a system is impossible. However, NIRS is validated in a number of studies [Skov *et al.* 1991, Wickramasinghe *et al.* 1992, Bucher *et al.* 1993] and can be considered as a reliable method reflecting cerebral oxygenation. In this study a comparison is made between a conventional NIRS instrument and the new INVOS instrument, in order to obtain information about sensitivity and usefulness of the INVOS system.

Changes in cerebral haemodynamics were induced by a moderate decrease of the arterial oxygen saturation (SaO_2) and by varying the arterial carbon dioxide level ($PaCO_2$), both by manipulating the inspired gas mixture. Changes in the $PaCO_2$ will result in a higher (hypercapnia) or lower (hypocapnia) cerebral blood flow [Strandgaard *et al.* 1992]. This should be reflected in both the NIRS signals and the INVOS signal. This study was performed with healthy volunteers to exclude pathophysiological changes of the cerebral circulation.

Materials and methods

Subjects

Eighteen healthy volunteers, 9 female and 9 male (mean age 56 years, range 21-83 years) participated in the study. The subjects had to abstain from caffeïnated drinks at least 2 hours prior to the experiment. None of the subjects were on medication. All subjects gave informed consent to participate in the study after full explanation of the research protocol, approved by the medical ethical committee of the Universitary Lung Centre Dekkerswald and the St. Radboud Hospital Nijmegen.

Cerebral oximetry

Conventional NIRS

This is a non-invasive and continuous optical method for measuring tissue oxygenation and haemodynamics. The technique is based on the relative transparency of biological tissue to light in the near infrared region (700-1000 nm) and on the existence within tissues of chromophores which are present in variable concentration and whose light absorbing properties vary with oxygenation. For cerebral tissue these chromophores are oxy- and deoxyhaemoglobin (O_2Hb and HHb) and, to a lesser extent, cytochrome oxidase, the terminal enzyme of the mitochondrial respiratory chain. The changes in absorption, measured at at least as many wavelengths as there are chromophores present, can be converted into changes in concentration [Delpy *et al.* 1988, Livera *et al.* 1991]. By using a "differential pathlength factor" (DPF), which corrects for the scattering of light within the tissue, the concentration changes can be quantitated [Wyatt *et al.* 1990a]. The concentration variables however will still start from an arbitrary base level.

The instrument (Radiometer Medical A/S, Denmark) is equipped with 4 sequentially pulsed laser diodes with wavelengths of 775, 805, 845 and 904 nm with a peak power of 10 W per pulse. The light from the laser diodes is coupled into a flexible fibre optic bundle (optode), attached to the (left) forehead. Some centimetres away a second fibre optic bundle is attached to the forehead to collect the transmitted light, detected by an avalanche photo diode. In this experiment a fixed

distance of 5.5 cm for the optodes was used. The absorption data were collected every second and displayed real-time on screen after having being converted into concentration changes.

INVOS

The INVOS system relies on the same principles as conventional NIRS. There are two major differences. Firstly light of 2 wavelengths (730 and 810 nm), emitted from light emitting diodes (LED), is used. Cytochrome oxidase can therefore not be detected. In physiological situations this is not a problem, as no large changes are to be expected in the cytochrome oxidase level [Ferrari *et al.* 1995]. Secondly the scattered light is received by two photodiodes which are placed at different distances from the light source (3 and 4 cm). This corresponds with two penetration depths of the photons emitted into the tissue. By processing the differential signal the influence of superficial (non-cerebral) changes in haemoglobin oxygenation should be reduced [McCormick *et al.* 1991, McCormick *et al.* 1992]. After processing the INVOS displays the regional cerebral oxygen saturation (rSO_2) as a percentage. All diodes are built into a disposable and flexible adhesive pad which can easily be fixed to the (right) forehead.

Instrumentation

All subjects were placed in a comfortable supine position, lying on a bed. Arterial oxygen saturation (SaO_2) was monitored continuously using a pulse oximeter (N200, Nellcor Inc., USA), with a reflection probe attached to the cheek to correct for temporal differences in circulation. End tidal CO_2 ($PetCO_2$) was monitored with a capnograph (N1000, Nellcor Inc., USA). The inspired oxygen fraction (F^IO_2) was monitored with an oxygen analyzer (OM-11, Beckman Inc., USA). The subjects were breathing in a closed spirometer system, in which the O_2 and CO_2 concentrations could be controlled. For safety reasons the ECG was monitored. All data were collected at a rate of 1 Hz for off-line analysis.

Protocol

The protocol was divided in four stages. During the first stage the subjects were placed in supine position and the various sensors were attached and the mouthpiece was connected. Steady state baseline values were obtained after 15 min.

During the second stage the F^IO_2 was lowered until it resulted in a SaO_2 of between 80 and 90%, equivalent to an altitude of 4-5 km. During the third stage CO_2 was slowly added to the closed breathing circuit until the $PetCO_2$ was increased by approximately 1 kPa. During the last stage the subjects were asked to hyperventilate at a frequency twice the breathing frequency at rest. The subjects were guided by a metronome. Visual feedback on the tidal volume was obtained directly from the

spirometer in the closed system. Hyperventilation was stopped after the $PetCO_2$ had decreased approximately 1 kPa. During the first, third and fourth stage an arterialized capillary blood sample was taken from the finger to measure $PaCO_2$, after the hand was warmed in water of 40 °C. Directly after withdrawal the analysis of the blood sample was performed on a blood gas analyzer (IL1312, Instrumentation Laboratory, Italy)

Analysis

All INVOS data were compared with both SaO_2 and with the difference between O_2Hb and HHb, named HbDiff. Due to the high variation in baseline values of rSO_2, we took for the INVOS data the *change* from the baseline value for comparison with the other signals. Linear regression analysis was performed and the Pearson correlation coefficient calculated between SaO_2 as independent and rSO_2 as dependent variable and HbDiff as independent and rSO_2 as dependent variable. All values are given as mean ± S.D.

Results

The baseline values for rSO_2 and SaO_2 after the 15 min steady state were 69.8 ± 6.2% (range 59-79%) and 98.6 ± 1.2% (range 96-100%) respectively. There was no correlation with age or gender. In Figure 3.1 the relationship between the maximum change from baseline value of rSO_2 and SaO_2 during the 2 desaturations is given ($p=0.005$). The regression line is given by $\Delta rSO_2\ (\%) = 0.82 \cdot \Delta SaO_2 + 9.5$. The correlation coefficient coefficient is 0.47. For one subject, were the desaturation was voluntarily more than average, the continuous relationship between both rSO_2 and the change in HbDiff, and SaO_2 is shown in Figure 3.2.

During hypercapnia the average $PaCO_2$ increased by 0.62 ± 0.26 kPa. The SaO_2 showed a small but insignificant increase of 0.9 ± 1.3%. In Figure 3.3 the relationship between the change in rSO_2 and HbDiff is given ($p=0.006$). The regression line is given by $\Delta rSO_2\ (\%) = 0.82 \cdot \Delta HbDiff + 0.20$. The correlation coefficient is 0.62.

During hypocapnia the average $PaCO_2$ decreased by 0.77 ± 0.40 kPa. The SaO_2 increased by 0.9 ± 1.2%. In all subjects HbDiff decreased under influence of CO_2. The mean rSO_2 decreased by 1.7 ± 2.6%. In 4 subjects an increase in rSO_2 was found, in 1 subject no change was observed. This is shown in the upper left quadrant of Figure 3.3, where also the relationship between the change in rSO_2 and HbDiff during hypocapnia is given. Although the regression line is drawn in Figure 3.3, it is for the hypocapnic situation not significant ($p=0.28$).

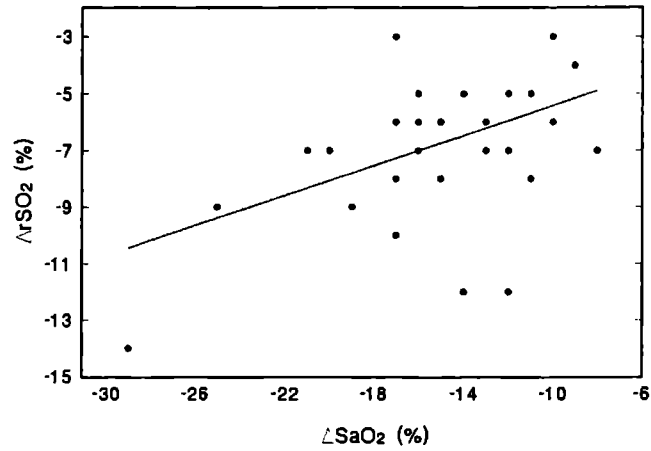

Figure 3.1 1 The relationship between the change from baseline value of the regional cerebral oxygen saturation (rSO_2) measured by the INVOS and the arterial saturation (SaO_2) measured by pulse oximetry during the 2 desaturations (n=35). Not all points are visible due to overlap.

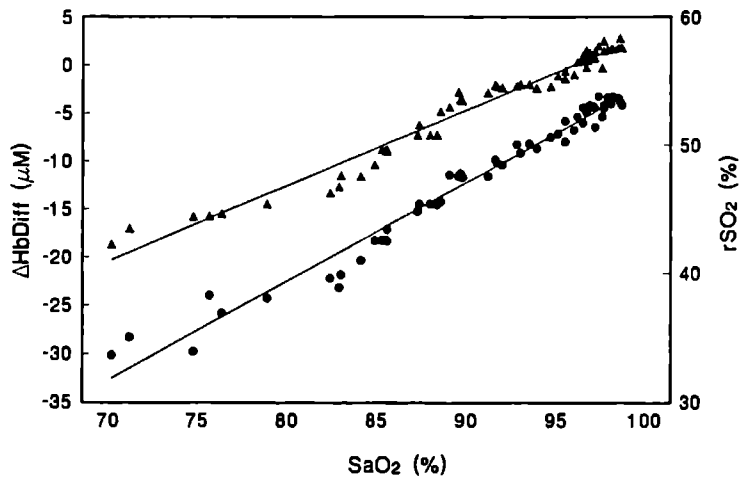

Figure 3.2 In this figure the relationship between the regional cerebral oxygen saturation (rSO_2) measured by the INVOS(▲) and the the change of oxy- minus deoxyhaemoglobin (HbDiff) measured with NIRS (●) compared to arterial saturation (SaO_2) measured by pulse oximetry is given for 1 subject undergoing a voluntarily more than average desaturation.

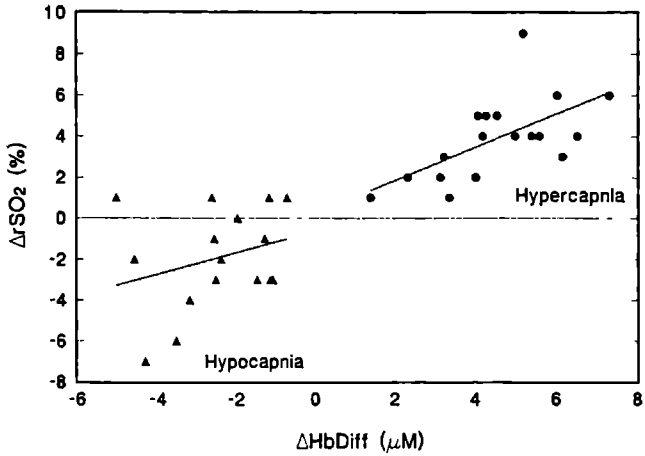

Figure 3.3 The relationship between the change from baseline value of the regional cerebral oxygen saturation (rSO_2) measured by the INVOS and the change of oxy- minus deoxyhaemoglobin (HbDiff) measured with NIRS during hypercapnia (●) and hypocapnia (▲).

Discussion

The INVOS showed a wide range in the displayed baseline values of rSO_2 (59-79%) in the healthy subjects used in this study. With a mean venous cerebral saturation of 62% [Gibbs *et al.* 1942] and a mean arterial SaO_2 of 99% this results in a mid-capillary SO_2 of 80%. All baseline values are lower, indicating that the rSO_2 is heavily influenced by the venous compartment of the brain [Mchedlishvili 1986]. The variability of the rSO_2 would indicate a large heterogeneity of the oxygen extraction in the brain. In this set of healthy subjects this seems not realistic. This makes that the displayed numerical information on the INVOS is a misleading illusory certainty.

The slight decrease in cerebral blood flow, induced by hypocapnia, consistently results in a decrease in HbDiff measured by conventional NIRS. The results of the INVOS however are inconsistently.

The increase of cerebral blood flow, induced by hypercapnia, is well displayed in both NIRS signals and INVOS signal. The correlation between ΔrSO_2 and ΔHbdiff during hypercapnia indicate that both systems measure saturation at tissue level, since the SaO_2, measured by pulse oximetry did almost not change. The correlation of only 0.62 can partly be explained from the displayed rSO_2 value, which changes in steps of 1%. This does not improve the accuracy of the system. In

cases were only small changes are to be expected this will result in a poor correlation, as can be observed during hypocapnia were it results in inconsistent observations.

In all measurements the INVOS instrument shows both a delay of a few seconds and a high degree of averaging in the algorithm. This makes the system not suitable to monitor patients in whom short but transient cerebral hypoxaemia could be expected.

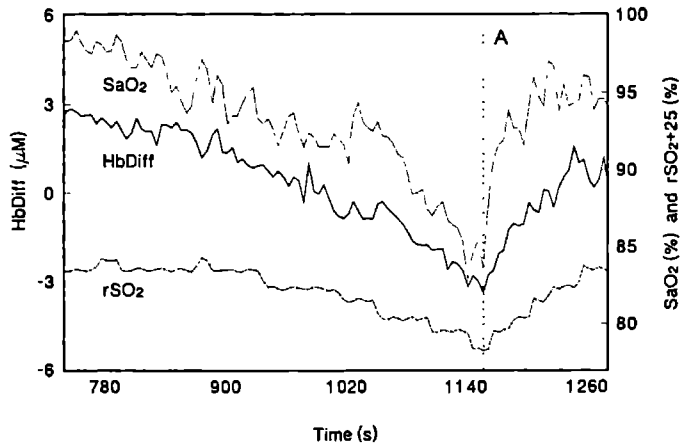

Figure 3.4 The arterial saturation (SaO_2) measured by pulse oximetry, the regional cerebral oxygen saturation (rSO_2) measured by the INVOS and the change of oxy- minus deoxyhaemoglobin (HbDiff) measured with NIRS as a function of time for 1 subject during a gradual decrease of the inspired oxygen concentration. At point A the desaturation is ended.

Conclusion

In literature both positive [Williams *et al.* 1994] and negative [Harris *et al.* 1993, Brown *et al.* 1993] studies concerning the INVOS are published. We consider the concept of the INVOS as positive but the currently existing version does not have the potential to be a useful addition to nowadays available clinical monitoring systems. The numerical information displayed by the INVOS does not provide any additional information to conventional NIRS, in which only relative changes of separate signals are displayed. Conventional NIRS has the advantage of fast and consistent trend information.

Measurement of the blood supply to the abdominal testis by means of Near Infrared Spectroscopy

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(Eur Urol 1995; 27: 160-166)

Summary

Cryptorchidism is the most common male sexual disorder. In case of an abdominal testis there is no objective criterion to choose between autotransplantation or orchiopexy after ligation of the spermatic vessels with subsequent development of collateral blood supply. By combining near infrared spectroscopy (NIRS) with pulse-oximetry the active testicular blood volume (ATBV) before and after occlusion of the spermatic vessels can be calculated in an animal model. NIRS is a non-invasive continuous optical technique that measures tissue oxygenation and haemodynamics. Ten boars with one non-palpable testis were selected. The spermatic vessels and vas deferens were separately prepared and atraumatic occluders were placed around the vessels. ATBV was measured before and after occlusion of the spermatic vessels. The calculated ATBV was 18.3 ± 2.3 ml/100g of testicular tissue, not corrected by division by the pathlength factor, accounting for light scattering in the tissue. In 5 out of 10 boars no significant ATBV was found after occlusion of the spermatic vessels, suggesting subsequent atrophy. NIRS combined with pulse-oximetry provides us with reproducible quantification of ATBV. The method can be used to investigate the viability of a testis after (temporary) occlusion of the spermatic vessels.

Introduction

Cryptorchidism is the most common male sexual disorder with an incidence of about 1% at the age of one year. Patients with an intra-abdominal testis pose diagnostic and therapeutic problems to the urologist: which technique of orchiopexy gives the best results? Autotransplantation [Frey *et al.* 1989, Silber 1981] gives good results, but is time consuming, painful and quite difficult at the age of 1 to 2 years. More recently a two-staged orchiopexy with clipping of the spermatic artery and vein with subsequent development of the collateral blood supply via the vas deferens is advocated [Steinhardt *et al.* 1985, Ransley *et al.* 1984]. Although there seems to be little atrophy after clipping of the testicular vessels, long term results are not yet fully known [Cendron *et al.* 1993]. The first stage (clipping of the spermatic vessels) and even the second stage (orchiopexy) can now be done laparoscopically in day-care [Bloom 1991, Elder 1989, Froeling *et al.* 1994].

At the time of surgery there is no objective and readily available test to predict if there will be sufficient blood supply for maintaining adequate testicular function and preventing atrophy. A 5 minute bleeding test can be performed [Stephens 1988], which cannot be considered to be an objective test. One study has proposed the use of a laser doppler flow meter for the perioperative measurement of testicular blood flow [Harrison *et al.* 1991]. This technique however has a limited penetration depth of about 1 mm and thus measures only the superficial blood flow and not the blood flow in the testis as a whole.

After clipping the spermatic vessels a certain fraction of the blood within the testis becomes stagnant and does not contribute any more to the oxygenation of the

tissue. The supply of oxygen, if any, is then only provided by the collateral circulation via the vas deferens. The circulating blood volume in the testis can be calculated by measuring the amount of circulating oxyhaemoglobin (O_2Hb) and deoxyhaemoglobin (HHb). The result is called the active testicular blood volume (ATBV). Determination of the ATBV before and after occlusion of the testicular vessels could provide us with an objective measurement to investigate the viability of the testis. We have developed an animal model in which reproducible results of the measurement of ATBV were obtained using transillumination of the testis by near infrared spectroscopy (NIRS) combined with pulse oximetry for quantification.

Materials and methods

Principles of NIRS

NIRS is a non-invasive and continuous optical method for measuring tissue oxygenation and haemodynamics. It was first described by Jöbsis in 1977 and further developed in the eighties [Rea *et al.* 1985, Cope *et al.* 1988]. Currently it is mainly used for monitoring the cerebrum [Brazy 1991, Wickramasinghe *et al.* 1993].

The technique of NIRS is based on two fundamental characteristics: the relative transparency of tissue to light in the near infrared region and the oxygenation dependent absorption changes in the tissue caused by chromophores, mainly O_2Hb and HHb. By measuring changes in absorption at different wavelengths tissue oxygenation can be monitored continuously. The relation between absorption and concentration is given by a modified Lambert-Beer law [Delpy *et al.* 1988], in which a pathlength factor is incorporated which accounts for the light scattering in the tissue. This factor is known for a limited number of tissue samples and not yet for testicular tissue.

By combining the NIRS data with pulse oximetry data it is possible to calculate an absolute value for blood volume in the examined tissue. This method is fully discussed by Wyatt *et al.* [1990] for the estimation of cerebral blood volume. We have used it to calculate testicular blood volume. Briefly, the effect of a small, gradual and transient change in arterial saturation (SaO_2) on the O_2Hb concentration is monitored. A decrease in SaO_2 of approximately 10 to 15%, induced by lowering the inspired oxygen concentration, is sufficient to calculate the blood volume. Provided that in the testis blood flow, volume and oxygen consumption remain constant during the procedure of desaturation the (absolute) change in arterial saturation is compared to a (relative) change in concentration of O_2Hb. From this the testicular blood volume can be calculated.

Animals and instrumentation

We selected ten young boars, body weight 15-25 kg, with one non-palpable testis from neighbouring pigfarms. The boars were premedicated with azaperone (2 mg/kg i.m.; Stresnil: Janssen Pharmaceutica, Belgium). Anesthesia was induced with metomidate (4 mg/kg i.v.; Hypnodil: Janssen Pharmaceutica, Belgium) and sodium pentobarbital (3 mg/kg i.v.; Narcovet: Apharmo, The Netherlands).

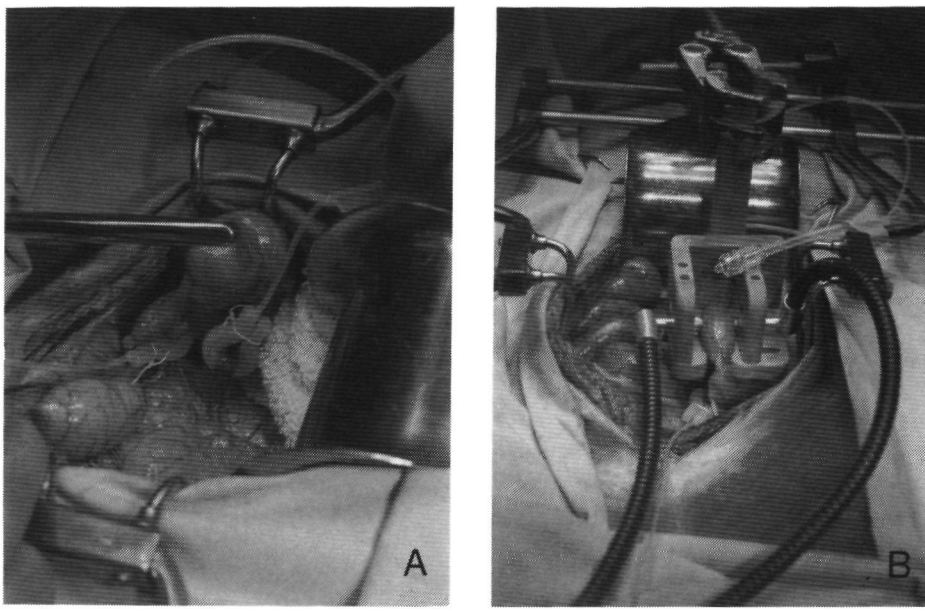

Figure 4.1 Experimental setup for the detection of ATBV in the testis of a boar. **a** Atraumatic inflatable occluders are placed around the vas deferens and its collaterals (left) and the spermatic vessels (right). **b** The testis is shown in the holder with attached to it the optical fibres

The animals were intubated and mechanically ventilated with a mixture of oxygen and nitrous-oxide (1:4) together with 0.8% enflurane (Ethrane, Abbott Medical, The Netherlands) to maintain anesthesia. The inspired oxygen concentration, end tidal CO_2 and enflurane concentration were monitored with a Capnomac (Datex, Finland). The carotid artery was cannulated to obtain arterial blood samples. Directly after withdrawal of the sample, analysis was performed on a blood gas analyser and oximeter (Instrumentation Laboratory IL1312 and IL482, Italy). A 4-wavelength NIRS instrument (Radiometer Medical A/S, Denmark) was used. A pulse oximeter (Nellcor N200, USA) measured SaO_2 continuously with a reflection probe attached to either ear or nose. A lower midline laparotomy was

performed. In all boars the non-palpable testis was found in the abdomen (6 testes at the left and 4 at the right side) approximately 5 centimetres from the internal inguinal ring. The position of the testis and the origin of the vasculature was similar in all cases. The spermatic and deferential artery both originated from the descending aorta. The corresponding veins ended in the inferior caval vein [Popesko 1963]. Apart from the spermatic vessels and the vas deferens there were no other visible vessels supplying the testis. The size of the testis was measured. The spermatic vessels and the vas deferens with its vessels were carefully dissected. Atraumatic inflatable occluders (3 or 5 mm, Rhodes Medical Instruments, USA) were applied around the spermatic vessels and around the vas deferens with its vessels (Figure 4.1). The testis was placed without torsion to the vessels in a holder to which the optical fibres of the NIRS instrument were attached. Heart rate and ECG were monitored continuously. An average heart rate of between 70 and 120 beats/minute was accepted as physiologically normal. The body temperature of the animal was kept constant with a heating pad. The testis was humidified and kept at constant temperature using a thermostated saline infuser system. All data were stored on a fixed disk for off-line analysis and calculation of ATBV.

Experimental protocol

After a stabilisation period we first measured the ATBV without any intervention. At least two measurements were done. An ATBV measurement was rejected if the heart rate changed beyond the physiological range or the ATBV itself changed more than 15% of the change of O_2Hb during the manoeuvre. Figure 4.2 illustrates a typical response to an ATBV measurement. Figure 4.3 illustrates the off-line calculation of the ATBV from the traces of Figure 4.2. Secondly the ATBV was measured during occlusion of the spermatic vessels. After a stabilisation period the procedure was repeated. If desaturation did not influence the NIRS signals, indicating no significant ATBV, the procedure was not repeated. If during desaturation an ATBV was measured it would prove the existence of significant collateral blood flow. Thirdly the atraumatic occlusion of the spermatic vessels was released and after stabilisation the vas deferens together with its vessels were occluded. If a clear change in the haemoglobin traces was found the ATBV was measured again with the procedure of desaturation. During the off-line analysis the signals were corrected for temporal between differences cerebral and peripheral circulation. An absolute ATBV could not be calculated as the pathlength factor for testicular tissue is not yet known. For obtaining an absolute value for the ATBV our results have to be corrected by this pathlength factor. This however is not necessary for a comparison of the measurements before and after occlusion of the vessels. In these measurements the pathlength factor is an unknown, but constant factor.

The distance between the optical fibres of the NIRS instrument was measured. An estimation of the volume was made by measuring length, height and width of the testis and assuming it has the shape of an ellipsoid. The specific density, needed for the calculation of ATBV, was determined from a control group of abdominal testes in boars. This was done by measuring weight, and volume by immersion in a graduated cylinder of saline. For statistical analysis Wilcoxon's signed rank test was used.

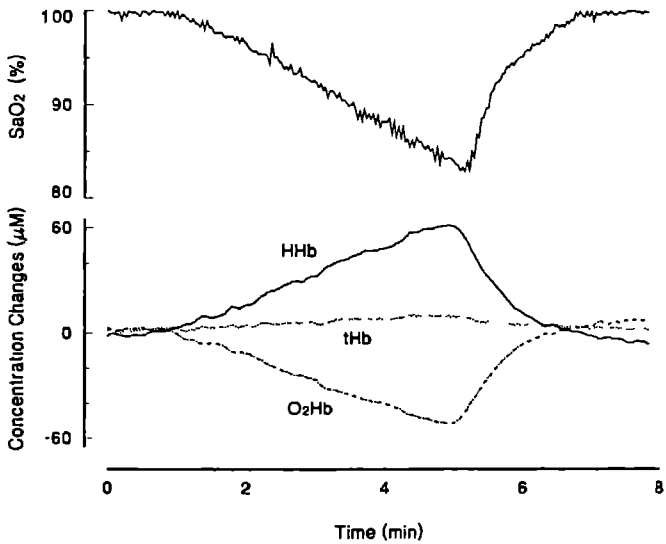

Figure 4.2: The pulse oximeter (upper trace) and NIRS (lower trace) signals during a gradual and transient deoxygenation. Notice the stable tHb signal, indicating that the ATBV stays constant during the manoeuvre and is not influenced by the deoxygenation. The signals have been corrected for the temporal difference between the circulation to the head and to the abdominal area.

Results

The mean volume of the testes was 6.0 ± 1.1 cm^3. The specific density was 1.05 g/cm^3. The average ATBV as supplied by the spermatic vessels as well as the vas deferens with its vessels was 18.3 ± 2.3 ml/100g testicular tissue. After occlusion of the spermatic vessels in 5 out of 10 animals no significant ATBV was found, which means that there is no circulating oxygenated blood present in the testis at that moment. In the other 5 animals an ATBV of 13.6 ± 3.2 ml/100g testicular tissue was

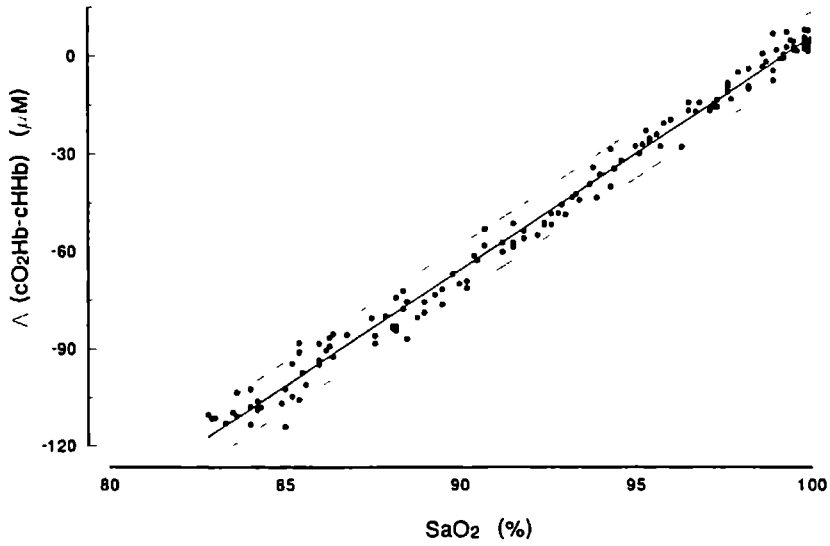

Figure 4.3 Relation between SaO_2 and NIRS data. The change in concentration of (O_2Hb-HHb) is plotted against SaO_2. Only the data points of the decreasing part of saturation signal in figure 4.2 are used for the calculation. The regression line is given by $\Delta(cO_2Hb\text{-}cHHb) = -709 + 7.2 \cdot SaO_2$ with a regression coefficient of 0.995. Confidence bounds of 95% are drawn in the graph

found, which means there is circulating oxygenated blood present. Before occlusion the average ATBV in these five animals was 14.6 ± 2.3 ml/100g testicular tissue.

Occlusion of only the vas deferens and its vessels did not alter the amounts of O_2Hb and HHb in any of the 10 animals and thus the oxygenation of the testes.

The individual values for ATBV as well as testis volume are given in Table 4.1. All the values for ATBV still have to be divided by the pathlength factor of testicular tissue. Table 4.2 gives the changes in concentration of the haemoglobin signals during occlusion of the spermatic vessels and during occlusion of the collaterals.

Discussion

The pulse oximetry sensor for measurement of the arterial saturation is placed on the head of the animal whereas the NIRS signals are derived from the testis. This means that there is a time delay between blood passing the two sensor positions. This delay time becomes effective during changes in oxygenation. The oxygenated blood first passes the head before it reaches the abdominal area. To prevent errors in the calculation of ATBV it is necessary to synchronise NIRS and pulse oximetry signals. The time delay can be obtained from the well defined, sharp change in both signals which occurs during fast re-oxygenation.

Table 4.1 Individual results of testis volume and of the calculation of the ATBV before and after occlusion of the spermatic vessels. Averages given as mean ± SEM. The value of the ATBV per animal with a percentage error presenting the maximum scatter of the interindividual ATBV measurement. Values of ATBV are not corrected for the path length factor, accounting for light scattering.

Animal	*Testis volume* (cm^3)	*ATBV before occlusion* (*ml/100g*)	*ATBV after occlusion* (*ml/100g*)
1	4.8	20.2 (±10%)	23.1 (±10%)
2	5.5	19.5 (±11%)	16.7*
3	7.2	13.5 (±12%)	15.0 (±4%)
4	5.4	10.7 (±12%)	8.1 (±23%)
5	7.0	9.1 (±14%)	5.3 (±17%)
6	14.8	23.8 (±19%)	NS
7	4.0	30.9 (±13%)	NS
8	5.8	14.1*	NS
9	3.5	13.4 (±15%)	NS
10	2.1	28.0 (±5%)	NS
Average	6.0 (1.1)	18.3 (2.3)	13.6 (3.2)

NS: No significant ATBV
* Single measurement

Table 4.2 Changes in concentration of O_2Hb, HHb and tHb (mean ± S.E.M) in the testis of all ten animals as an effect of occlusion of the spermatic vessels or of the vas deferens and its collaterals. Units are μM and not corrected for the pathlength factor.

Concentration changes (μM)	*Δ(HHb)*	*Δ(O_2Hb)*	*Δ(tHb)*
Occlusion of spermatic vessels	116 (26)	-57 (13)	58 (14)
	($p < .002$)	($p < .002$)	($p < .004$)
Occlusion of vas deferens and its collaterals	3.0 (1.6)	-1.3 (1.3)	1.7 (0.7)
	NS	NS	NS

NS: Not Significant

In the example of Figure 4.2 the desaturation procedure started with an SaO_2 of 99% and continued until a level of 85%. The tight correlation between both signals, as shown in Figure 4.3, indicates that a temporary decrease in oxygen saturation of less than 10% is sufficient to obtain reliable results. Such a small and transient deoxygenation can safely be administered to patients.

Since the actual pathlength factor of testicular tissue is not known the calculated values of the ATBV are not corrected for it. This means that the actual ATBV can be

estimated only if we assume a value for the pathlength factor. In comparison with other tissues we expect the pathlength factor for testicular tissue to be within the range of 4.5 to 5.5. This value is in line with the value for neonatal head and adult head or muscle tissue [Essenpreis *et al.* 1993]. Although in principle not difficult to measure, the exact determination of the pathlength factor requires expensive optical equipment with picosecond time resolution which is only available to a limited number of research groups. For our application however the pathlength factor is not so important as we compare the calculated value of ATBV before and after occlusion of the spermatic vessels in which case the pathlength factor remains constant. If measurements are to be compared with a time difference of several months one should take into consideration that the pathlength factor might change slightly due to histological changes within the testis, e.g. by atrophy or growth.

The individual results of the ATBV as shown in Table 4.1 were reproducible in all ten animals. If we assume a pathlength factor of 5, an average absolute value of ATBV of 3.7 ± 0.5 ml/100g testicular tissue is obtained, which is in the physiologically expected range. The inter-individual ATBV varies from 9.1 to 30.9 ml/100g testicular tissue (uncorrected for pathlength factor). One explanation for this could be a variation in this factor. Essenpreis *et al.* [1993] found a substantial variation between individuals in the pathlength factor for the adult head, neonatal head and the adult calf. This is closely related to tissue composition which in the testis is relatively uniform and not influenced by skin or bones. Another explanation can be the biological variability in vascularisation of the testis [Desjardins 1989]. The variation in ATBV that we find shows similarities with the observations in the determination of cerebral blood volume in newborns [Wyatt *et al.* 1990].

In all testes we have found an increase in total haemoglobin concentration, which is the sum of O_2Hb and HHb and similar to blood volume, during the occlusion of the spermatic vessels. We believe this is due to the influx of blood via the vas deferens and its collaterals. Once the arterial blood enters, via anastomoses, the spermatic circulation part of it becomes stagnant and no longer contributes to the oxygenation of the testis. This might be of clinical importance.

In some animals we found slow oscillations in the HHb and O_2Hb signals (Figure 4.4) which could not be correlated to respiration, heart rate or irrigation of the testis. In two cases the magnitude of the oscillations disturbed the determination of the ATBV (see Table 4.1). The periodicity of the oscillations was 40-50 seconds. We believe these oscillations are Mayer waves due to vasomotor activity [Preiss *et al.* 1974]. We cannot explain why this phenomenon occurs in some animals while in others this effect is not seen. A possible explanation might be the cannulation of the carotid artery for obtaining blood samples, since manipulation in the carotid region can lead to oscillations.

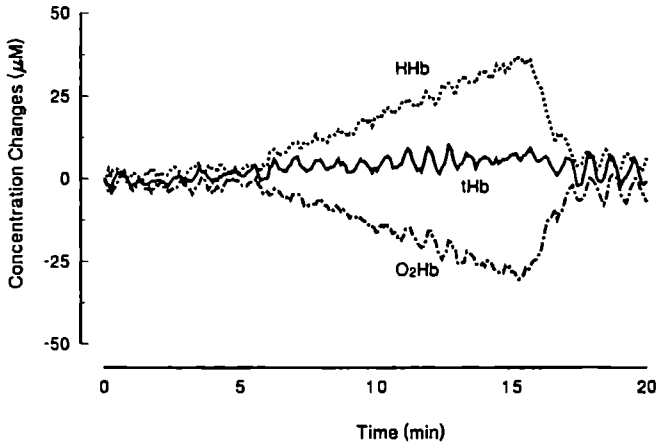

Figure 4.4 During desaturation oscillations in the O_2Hb and HHb signals were found. The periodicity of the oscillations in this graph is 48 seconds. We believe the oscillations are due to vasomotor activity, so called Mayer waves.

The fact that 5 out of 10 boars showed no ATBV after occlusion of the spermatic vessels suggests that these testes will become atrophic after ligation and immediate orchiopexy. This might explain why the reported successrate (no atrophy) of the one-stage Fowler-Stephens procedure varies from 50 to 81% [Boddy *et al.* 1991, Gibbons *et al.* 1979]. In a two-stage operation the reported successrate is 86% [Bloom 1991].

This method can be used to determine intra-operative whether there remains sufficient collateral blood supply to the testis after occlusion of the spermatic vessels. If this is positive a (laparoscopic) two stage or even one stage Fowler-Stephens procedure can be performed. If there seems to be no significant ATBV an autotransplantation needs to be considered.

Currently the method is used to predict testicular viability after clipping of the spermatic vessels with subsequent inspection after 2½ months. The technique could be adapted for laparoscopic use, allowing a minimally invasive diagnostic and possibly therapeutic procedure. Follow up studies will be needed to measure the development of collateral blood supply in the testis after ligation of the spermatic vessels.

Conclusion

NIRS is an excellent and reproducible tool for determination of ATBV of an intra-abdominal testis. In 5 out of 10 boars no circulating oxygenated blood was present in

the testis after occlusion of the spermatic vessels, suggesting subsequent atrophy. The technique of measuring ATBV can be used as an objective tool to investigate if the presence of ATBV is related to testicular viability after ligation of the spermatic vessels.

Acknowledgements

We would like to thank Jos Evers for his biotechnical assistance during the experiments and Theo Arts for his help in the search for young boars with a congenital abdominal testis.

Prediction of tissue viability in abdominal testis using Near Infrared Spectroscopy

Frank M.J.A. Froeling, Willy N.J.M. Colier, Berend Oeseburg and Jos D.M de Vries

(Submitted)

Summary

Spermatic vessel ligation and orchiopexy of an abdominal testis sometimes results in testis atrophy. There is no test to predict testis viability after spermatic vessel occlusion. This problem is recognised in other types of surgery where tissue is mobilised. By combining near infrared spectroscopy (NIRS), a non-invasive continuous optical method, with pulse oximetry the circulating, active testicular blood volume (ATBV) can be determined before and after spermatic vessel occlusion. With these results long term viability after ligation was predicted. Atraumatic spermatic vessel occluders were placed in 14 boars with an abdominal testis. ATBV was measured before and after occlusion. In 6 of the 14 boars no ATBV was measured after spermatic vessel occlusion. The spermatic vessels of 9 boars were permanently ligated. After 2½ months these boars were operated again and the ATBV was measured. Of the 9 boars one died and was lost for follow-up. After 2½ months 5 testes were viable and 2 atrophic, as was predicted. One testis that had shown a dubious ATBV was swollen and necrotising. NIRS combined with pulse oximetry provides safe and reproducible measurements of ATBV. Testis viability after permanent spermatic vessel ligation was correctly predicted. The technique could be applied to examine tissue viability in other types of (pedicled) surgery.

Introduction

Spermatic vessel ligation in young boys with an abdominal testis can lead to testis atrophy [Ransley *et al.* 1984, Bloom *et al.* 1991, Cendron *et al.* 1993]. The spermatic vessels are ligated and cut in order to allow full mobilisation of the testis. The mobilisation and orchiopexy itself are delayed until a few months later during which collateral circulation can develop via the vas deferens (Fowler-Stephens procedure) [Stephens 1988]. Currently there is no readily available test that can predict testis viability after occlusion of the spermatic vessels. In case of possible atrophy after occlusion autotransplantation of the abdominal testis is the preferred technique. Also in other types of pedicled surgery (e.g., bowel, skin, muscle or kidney) the assessment of tissue viability can be a problem during mobilisation. To solve this problem various techniques have been explored, like laser Doppler flowmetry [Harrison *et al.* 1991, Hallock 1992], impedance monitoring [Harrison *et al.* 1989], polarographic P_{O_2} measurements [Kramm *et al.* 1989] and transcutaneous oxygen or carbon dioxide monitoring [Slagsvold *et al.* 1989, Rochat *et al.* 1993]. These methods however measure either only superficially, indirectly or perform spot measurements.

In this study near infrared spectroscopy (NIRS) was used, which can overcome these problems. NIRS is a non-invasive and continuous optical method measuring tissue oxygenation and haemodynamics. The method is based on the relative transparency of tissue for light in the infrared region and on the oxygenation dependent absorption spectra of oxyhaemoglobin (O_2Hb) and deoxyhaemoglobin (HHb). The method has a penetration depth of several centimetres. It is clinically in

use for measuring cerebral oxygenation in neonates [Wyatt *et al.* 1990b, Liem *et al.* 1994]. By combining NIRS with pulse-oximetry data it is possible to calculate values of circulating, and thus active testicular blood volume (ATBV), in abdominal testes before and after occlusion of the spermatic vessels [Colier *et al.* 1995]. On the basis of these findings it could be possible to predict long-term viability.

Materials and Methods

Fourteen young boars with a non-palpable testis were selected from nearby pig-farms. Parts of the procedure have been described by Colier *et al.* [1995]. Under general anesthaesia a midline incision was performed in the lower abdomen. All non-palpable testes were found in the abdomen approximately 5 cm from the internal ring of the inguinal canal. In all boars the spermatic and deferential artery both stemmed from the descending aorta. There were no other visible vessels supplying the testis. The size of the testis was measured and the anatomy recorded. Atraumatic vessel occluders (3 or 5mm, Rhodes Medical Instruments, USA) were placed around the vas deferens and spermatic vessels. The testis was placed without any tension into a holder to which the lightguides of the NIRS instrument (Radiometer Medical A/S, Denmark) were attached. A pulse-oximeter (Nellcor N200, USA) measured continuously the arterial saturation (SaO_2) with a reflection probe attached to either ear or nose. Without any further intervention the SaO_2 was temporarily decreased by 10 to 15% by lowering the inspired oxygen fraction, in order to calculate the ATBV. For calculation of the absolute amount of blood in the testis the pathlength factor of the examined tissue, which accounts for light scattering, is required [Delpy *et al.* 1988]. The ATBV was measured twice, before and after occlusion of the spermatic vessels. Figure 1 shows an example of a registration of the NIRS signals after occlusion of the spermatic vessels. After release of the occlusion of the spermatic leash the vas deferens was occluded to see if any influence on the oxygenation of the testis could be observed. Based on the presence of ATBV a prediction was made whether the testis would survive permanent ligation. After successful measurements in the first animals the spermatic vessels of 9 boars were ligated with metal clips. The abdomen was closed and reopened approximately 2½ months later in order to examine the testis. Without further intervention the ATBV of the abdominal testis was measured.

All data are presented as mean ± S.E.M. For statistical analysis a paired t-test was used.

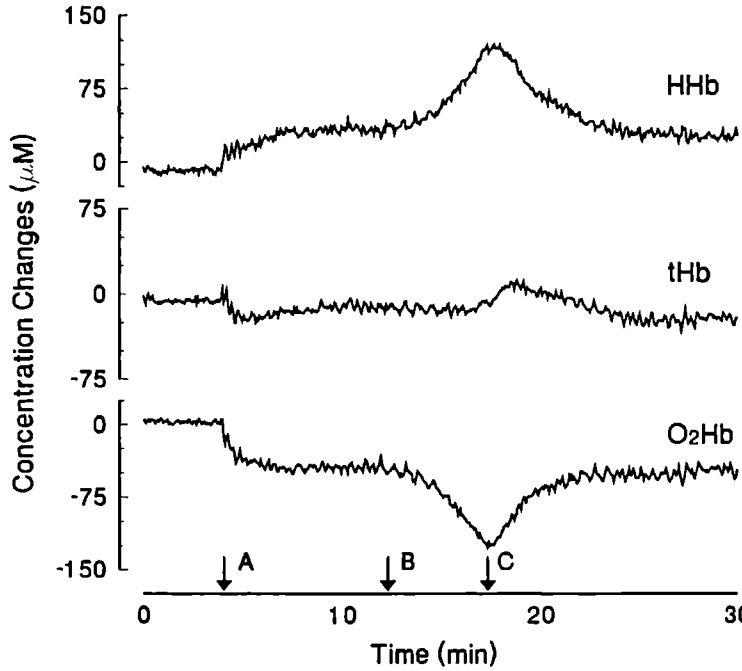

Figure 5.1 An example of a registration of NIRS signals. At point A the spermatic vessels are occluded. Point B is the start of the desaturation procedure. The decrease of the oxyhaemoglobin (O_2Hb) and the increase of the deoxyhaemoglobin (HHb) signal after point B indicates the presence of ATBV. The total haemoblobin (tHb) signal shows only a minor change. At point C the desaturation is ended.

Results

During the first operation the average volume of the abdominal testes was 6.2 ± 1.1 cm^3. Without intervention an average ATBV of 16.4 ± 1.9 ml/100g testicular tissue was measured. This value is not corrected by division by the pathlength factor. After occlusion of the spermatic vessels an ATBV of zero was measured in 6 boars, indicating no circulating blood at the time of examination. In the other 8 boars an ATBV of 13.7 ± 2.3 ml/100g was found. The average ATBV of these 8 animals *before* occlusion of the spermatic vessels was not significantly different (13.7 ± 1.6 ml/100g).

One of the 9 boars in which the spermatic vessels were permanently ligated died shortly after the first operation due to pneumonia. In the other 8 boars the

abdomen was reopened 76 ± 4 days after the first operation. In 5 boars a viable testis was found with an average ATBV of 15.7 ± 3.0 ml/100g. The volume had increased significantly from 5.8 ± 0.5 cm^3 to 15.2 ± 1.6 cm^3. All of these 5 testes had shown an ATBV of 15.2 ± 3.1 ml/100g after occlusion of the spermatic vessels during the first operation, which was not significantly different. Two testes had become atrophic. This was also in accordance with the previous findings that showed *no* ATBV after ligation during the first operation. In one boar a dubious ATBV was measured during the first operation. This testis was now swollen and necrotising.

Discussion

Currently there is no technique in use that can monitor oxygenation deep inside tissue during an operation. The available methods either examine the surface of tissue, like laser Doppler flowmetry, produce spot measurements within the tissue or provide only a crude circulation assessment, e.g. a 5 min bleeding test [Stephens 1988]. By combining NIRS with pulse-oximetry data during a small and transient decrease in central SaO_2 the ATBV can be calculated. A 10% decrease in SaO_2 during a short period is not considered hazardous for patients without cardio-pulmonary disease.

In Figure 5.1 at point A, after occlusion of the spermatic vessels, a small increase of the HHb and decrease of the O_2Hb signal are observed. If the oxygen consumption of the testis remains constant, this indicates a decreased arterial inflow. The diminished arterial inflow is compensated by a higher oxygen extraction within the testis, expressed in the increased HHb signal. The fact that after point B during desaturation changes in O_2Hb and HHb are still measurable, demonstrates the presence of circulating blood.

The calculated values of ATBV still need to be divided by the pathlength factor to obtain absolute values of ATBV. For testicular tissue this factor is not yet precisely known, but is estimated at approximately 5. In this test however the pathlength factor is not important because we relate measurements before and after ligation in which the pathlength factor is assumed constant.

One of the 9 boars that had permanent ligation of the spermatic vessels died of pneumonia one day after the operation. The interval was too short to assess the viability. For one testis conflicting data on the presence of ATBV were found. On the basis of these data the testis should have been viable. After 2½ months this testis was enlarged and necrotising, suggesting borderline viability. Obviously no immediate atrophy had occurred. For the other 7 testes the prediction was correct. An almost threefold increase in testicular volume in 5 boars within 2½ months indicates an adequate blood supply. The blood volume per 100g of tissue had not changed significantly during the 2½ months. The finding during the first operation

that only in 8 out of the 14 testes an adequate blood supply was measured might explain why the Fowler-Stephens procedure is not always successful [Ransley *et al.* 1984]. If the remaining blood supply in the group showing ATBV after occlusion of the spermatic vessels would not be further compromised during orchiopexy, e.g. due to stretching or torsion, the orchiopexy could be performed immediately.

Pulse oximetry in this study was needed to measure the SaO_2 of the animal and calculate afterwards values of ATBV. Currently the ATBV data are calculated off-line, but technically it is possible to do this real-time. For clinical use therefore NIRS combined with moderate arterial desaturation would suffice as a diagnostic tool to assess oxygenation within the tissue itself and predict viability. This would make the technique applicable for other types of pedicled surgery or transplants.

Conclusions

NIRS combined with pulse-oximetry data provides safe and reproducible measurements of circulating, active blood volume in testicular tissue. In a considerable number of abdominal testes no adequate blood supply was measured after occlusion of the spermatic vessels. Viability of the testis after permanent ligation was correctly predicted. The technique can be used to choose the suitable type of orchiopexy. Because this technique measures deep inside tissue a "viability monitor" is within reach for other types of surgery.

Determination of oxygen consumption in muscle during exercise using Near Infrared Spectroscopy

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(Acta Anaesthesiol Scand 1995; *in press*)

Summary

The aim of this study was to determine oxygen consumption (V_{O_2}) during isometric exercise in human muscles using near infrared spectroscopy (NIRS) The technique was used to study the relationship between V_{O_2} in the soleus muscle and the level of isometric exercise expressed as percentages of the maximum voluntary contraction (MVC) For the study 11 healthy male volunteers were recruited Reproducibility was studied in 6 subjects The subjects were seated in a chair with the knee joint at an angle of 90° The optodes of the NIRS instrument were attached to the lateral aspect of the soleus muscle A horizontal bar above the knee was connected to a dynamometer Subjects applied isometric force to the bar by producing a torque at the ankle joint Firstly the MVC was determined Secondly the V_{O_2} at rest and at 5 levels of isometric exercise, ranging from 5% to 25% of MVC and increasing by 5% each stage, was measured In all cases the V_{O_2} at rest or during isometric contraction was determined from the decrease of the oxyhaemoglobin (O_2Hb) signal immediately after arterial occlusion of the thigh Repeated measurements showed no significant difference between trials, indicating that the measurements were reproducible At rest a V_{O_2} of 6 7± 1 1 μM O_2Hb min^{-1} (mean ± S E M) was found, a result comparable with other studies In all subjects a linear relationship was found between the V_{O_2} and the level of exercise The average slope of the regression lines of all individuals was 0 85 ± 0 22 μMO_2Hb min^{-1} %MVC^{-1} (mean ± S E M) Inter-individual variation of the slopes was high and ranged from 0 28 to 2 29 μM O_2Hb min^{-1} %MVC^{-1}, which can be explained by differences in fat percentage and in the measuring volume of the NIRS instrument NIRS appeared to be a reproducible and reliable method for the non invasive measurement of V_{O_2} in human muscles The method could be used to investigate regional differences as well as changes in time between muscle groups as a function of training

Introduction

About 40% of the total body mass consists of skeletal muscle tissue. During exercise the major part of the total oxygen consumption (V_{O_2}) of the body takes place in these muscles. Respiratory gas analysis is the main method to measure V_{O_2} during exercise. In this way it is possible to evaluate overall aerobic power and follow the progress of a training protocol in time. This however gives no insight into regional differences in V_{O_2} of various muscle groups, which can be caused e g. by differences in muscle fiber type composition or by resistance to fatigue. Hartling *et al.* [1989] described a method of assessing regional V_{O_2}. The method was based on the measurement of blood flow to the muscle group and measurement of the arterio-venous difference in oxygen content. This method however has its drawbacks: it is invasive, inconvenient and not possible on a continuous basis during exercise. Our study therefore focused on the use of near infrared spectroscopy (NIRS) for assessing muscle V_{O_2}. NIRS is a non-invasive, continuous optical technique to measure tissue oxygenation, having none of the drawbacks of the method of Hartling *et al.* [1989].

Recently NIRS has been used to assess muscle $\dot{V}O_2$. Most of these studies were confined to the $\dot{V}O_2$ at rest [Cheatle *et al.* 1991], $\dot{V}O_2$ with a period of maximum voluntary contraction (MVC) [De Blasi *et al.* 1993], or recovery after a period of exercise [Hamaoka *et al.* 1992, Sahlin 1992]. The aim of this study was to investigate the relationship between $\dot{V}O_2$ as measured by NIRS and submaximal isometric force delivery, expressed as a percentage of MVC.

Materials and Methods

Subjects

Eleven healthy volunteers were recruited from a population of sporting, non-smoking males (aged 22-33 years). Six subjects underwent the experiment twice, where the second experiment took place on another day but at the same time of day, to test for reproducibility. The subjects refrained from caffeine and alcohol at least two hours before the experiment. All subjects gave informed consent.

NIRS

NIRS is a non-invasive and continuous optical method for measuring tissue oxygenation and haemodynamics [Jöbsis 1977, Wickramasinghe *et al.* 1993, Wyatt *et al.* 1990]. Currently it is mainly used to monitor (neonatal) cerebral oxygenation. In this study it was used to assess muscle tissue oxygenation. The technique is based on two fundamental characteristics: firstly the relative transparency of human tissue to light in the near infrared region and secondly the oxygenation dependent absorption changes in the muscle caused by chromophores, mainly oxy- and deoxyhaemoglobin (O_2Hb and HHb) and oxy- and deoxymyoglobin (O_2Mb and HMb). The sum of O_2Hb and HHb is a measure of the total blood volume (tHb) in the tissue. By measuring changes in light absorption at different wavelengths muscle oxygenation can be monitored continuously. The relation between the changes in absorption and concentration are given by a modified Lambert-Beer law [Delpy *et al.* 1988], in which a pathlength factor is incorporated which accounts for the light scattering in the muscle tissue.

Due to the overlap between the absorption spectra of myoglobin and haemoglobin it is not possible to distinguish between changes in concentration of O_2Hb and O_2Mb and HHb and HMb. This however is not significant for this study. Firstly, in human limbs the myoglobin contribution to the signal will be no greater than approximately 25% [Chance *et al.* 1991]. Secondly, considering the time course of our experiments there will be no significant deoxygenation of O_2Mb as this occurs after almost complete deoxygenation of O_2Hb [Wang *et al.* 1990a, Wang *et al.* 1990b].

The NIRS instrument (Radiometer Medical A/S, Denmark) used is an instrument with 4 wavelengths ranging from 775 nm to 904 nm. Data were sampled every second, displayed realtime and stored on disk. The algorithm used to convert absorption changes into concentration changes is described by Livera *et al.* [1991].

Set up

Figure 6.1 shows the experimental set up. The subjects were seated in a chair with the knee joint at an angle of 90°. A horizontal bar above the knee was connected to a dynamometer. In this set up the soleus muscle is the most active muscle, while the gastrocnemius muscle does not show any significant activity [Aratow *et al, 1993*]. Forces were measured with strain gauges (CA660, Peekel Instruments, The Netherlands) and registered on a paper recorder (BD 41, Kipp en Zonen, The Netherlands). A cuff was placed around the thigh and inflated pneumatically to a pressure of more then 270 mmHg to obtain a rapid arterial occlusion. During the course of the occlusion the cuff pressure was monitored with a manometer.

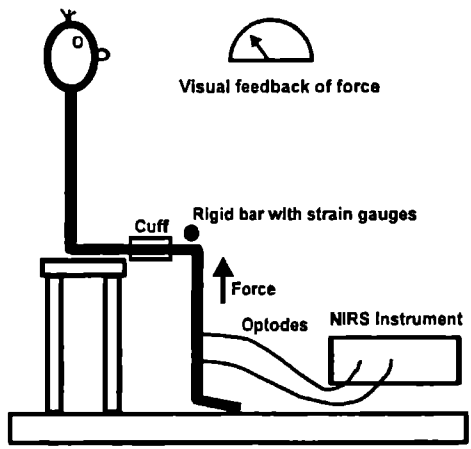

Figure 6.1 Experimental set up for the determination of the oxygen consumption ($\dot{V}O_2$) in the soleus muscle as a function of the maximum voluntary contraction. The $\dot{V}O_2$ is measured non-invasively using near infrared spectroscopy.

Two independent, slightly concave holders were fixed to the skin with doube-sided adhesive discs (No. 2181, 3M, USA). The holders were positioned on the lateral aspect of the right leg on the soleus muscle and below the transition of the gastrocnemius muscle into its distal tendon. This minimizes the contribution of the gastrocnemius muscle to the obtained NIRS signals. The optodes of the NIRS instrument were attached to the holders. The distance between the optodes was 4.5

cm in all experiments. For the muscle tissue a pathlength factor of 4.3 was used [Chance *et al.* 1988, Ferrari *et al.* 1992].

Protocol

Step 1: the isometric MVC was determined, being the highest force level applied to the bar during three consecutive plantar flexions. On the paper recorder percentages ranging from 5 to 25% by intervals of 5% were marked. The same MVC was used for the reproducibility test on the second day of the experiment.

Step 2: the $\dot{V}O_2$ at rest was determined during an arterial occlusion of 2 minutes.

Step 3: the $\dot{V}O_2$ was determined at 5 exercise levels, ranging from 5% to 25% of the MVC, increasing by 5% at each stage. The subjects started with isometric contraction and maintained the exercise for 2½ minutes. The subject had visual feedback from the percentage markings of the MVC on the paper recorder so they could maintain a constant level of exercise. Thirty seconds after the start of the isometric contraction the arterial occlusion was applied and maintained for 2 minutes. If a plateau in the deoxygenation was reached before the end of the 2 minutes the arterial occlusion was released and the isometric contraction stopped. No percentages of MVC higher than 25% were used to avoid local ischaemia due to an already restricted blood flow before inflation of the cuff [Barcroft *et al.* 1939].

Step 4: to check for changes in metabolism, the $\dot{V}O_2$ at rest was determined once more at the end of the trial during a 2 minute arterial occlusion.

Between each step of the protocol as well as between the different exercise levels of step 3 the subjects had a 5 minute rest period. In all cases the gradient of the decreasing O_2Hb signal immediately after occlusion was taken as the $\dot{V}O_2$ of the muscle. All the results of $\dot{V}O_2$ are expressed as micromoles O_2Hb per litre of tissue per minute. Since $\dot{V}O_2$ is also expressed as micromoles O_2 per 100 gram of tissue per minute a conversion factor is given. For this conversion we take into account the molecular ratio of haemoglobin to oxygen, which is 1:4, the density of skeletal muscle tissue, being 1.04 kg/l [Vierordt 1906] and division by 10 to go from kg to 100 g.

Statistical analysis

A linear regression analysis was performed and the Pearson correlation coefficient calculated between the percentage MVC as independent and $\dot{V}O_2$ as dependent variable. The measured $\dot{V}O_2$ at rest was compared to the intercept $\dot{V}O_2$ from the regression line with a paired t-test. The reproducibility was assessed by using a paired

t-test, comparing the slopes and intercepts of the individual regression lines during the first and second trial. Significance level was $p < 0.05$. Unless stated otherwise all results are reported as mean ± S.E.M.

Results

A typical NIRS tracing of exercising muscle with arterial occlusion is shown in Figure 6.2. At point A a force of 20% MVC was applied to the bar. The force was sustained and at point B the arterial occlusion started. The $\dot{V}O_2$ is calculated from the tangent of the O_2Hb signal. During the occlusion only a small increase in tHb can be observed. At point C no more force is applied and the arterial occlusion is released. After point C a hyperaemic reaction in the muscle can be observed.

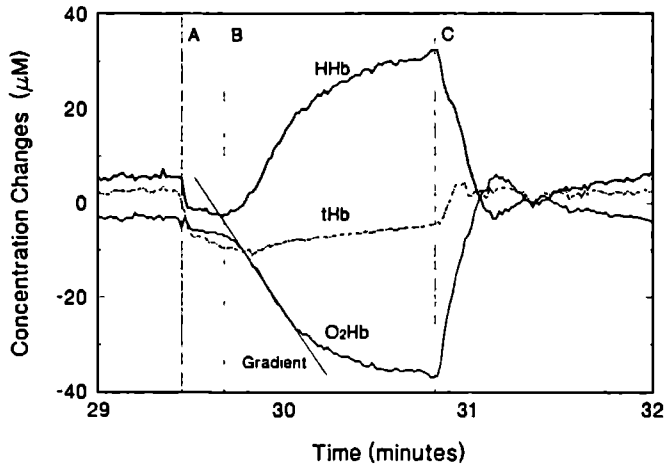

Figure 6.2 A typical tracing of the changes in the haemoglobin concentration measured with near infrared spectroscopy in exercising muscle for the determination of the oxygen consumption ($\dot{V}O_2$). At point A a force of 20% MVC was applied to the bar. The force was sustained and at point B the arterial occlusion started. The $\dot{V}O_2$ is calculated from the gradient of the O_2Hb signal. At point C no more force is applied and the arterial occlusion released.

The repeated experiments showed no significant differences between subjects (slope: $p=0.18$, intercept: $p=0.20$, $n=6$) indicating that the measurements were reproducible. In Table 6.1 the individual results for MVC, rest $\dot{V}O_2$ at the start and at the end of the experiment, and the slopes and intercepts of the regression lines of all subjects are given. The average MVC produced by the subjects was 893 ± 80 N. The rest $\dot{V}O_2$ at the start of the experiment (6.7 ± 1.1 $\mu M\ O_2Hb \cdot min^{-1}$ or 2.6 ± 0.4 $\mu mol\ O_2 \cdot min^{-1} \cdot 100g^{-1}$) was significantly different ($p < 0.02$) compared to the

rest $\dot{V}O_2$ at the end of the experiment (5.6 ± 1.0 $\mu M\ O_2Hb \cdot min^{-1}$ or 2.2 ± 0.4 $\mu mol\ O_2 \cdot min^{-1} \cdot 100g^{-1}$).

Table 6.1 The individual results of the Maximum Voluntary Contraction (MVC) and oxygen consumption ($\dot{V}O_2$) at rest measured at the start and at the end of the experiment and the calculated slopes and intercepts, including correlation coefficient and p value, of the regression lines of all subjects. Values are given as mean ± S E M

Subject	*MVC (N)*	*rest* $\dot{V}O_2$ *(start)* ($\mu MO_2Hb\ min^{-1}$)	*rest* $\dot{V}O_2$ *(end)* ($\mu MO_2Hb\ min^{-1}$)	*Slope* ($\mu MO_2Hb\ min^{-1} \cdot\ \%MVC^{-1}$)	*Intercept* ($\mu MO_2Hb\ min^{-1}$)	*Correlation coefficient*	*p value*
1	794	3 6	3 6	0 446	4 2	98	0004
2	1078	11 3	11 2	1 27	8 9	99	0002
3	569	5 0	3 5	0 405	5 5	99	0001
4	1147	11 7	10 7	0 642	11 4	99	0002
5	549	5.4	4 2	0.276	13 4	99	0008
6	1157	9 6	8 0	2 11	38 3	99	002
7	814	4 0	2 9	0 835	4 8	99	0002
8	1137	12 0	7 5	2 29	22 2	91	02
9	765	2 8	2 5	0 339	2 8	95	005
10	1235	5 0	4.3	0 383	5 9	98	0008
11	578	3 3	3 1	0 325	4 1	98	0004
Mean	893 ± 80	6 7 ± 1 1	5 6 ± 1 0	0 85 ± 0 22	11 0± 3 2		

In all subjects a linear relationship between relative force level and $\dot{V}O_2$ was found. The equation of $\dot{V}O_2$ as a function of the relative force level is given by $\dot{V}O_2$ ($\mu M\ O_2Hb \cdot min^{-1}$) = 0.85·%MVC + 11.0 or, in different units, by $\dot{V}O_2$ ($\mu mol\ O_2 \cdot min^{-1} \cdot 100g^{-1}$) = 0.32·%MVC + 4.2. This equation is derived from the averaged slopes and intercepts of the individual regression lines. For a graphical presentation (Figure 6.3) the individual values of $\dot{V}O_2$ were averaged per percentage MVC and at rest. This gave a not significantly different equation:
$\dot{V}O_2$ ($\mu M\ O_2Hb \cdot min^{-1}$) = 0.91·%MVC + 9.4 or
$\dot{V}O_2$ ($\mu molO_2 \cdot min^{-1} \cdot 100g^{-1}$) = 0.35·%MVC + 3.6. No significant difference was found between the measured rest $\dot{V}O_2$ at the beginning of the experiment and the rest $\dot{V}O_2$ derived from the intercept of the individual regression lines (columns 3 and 6 in Table 6.1).

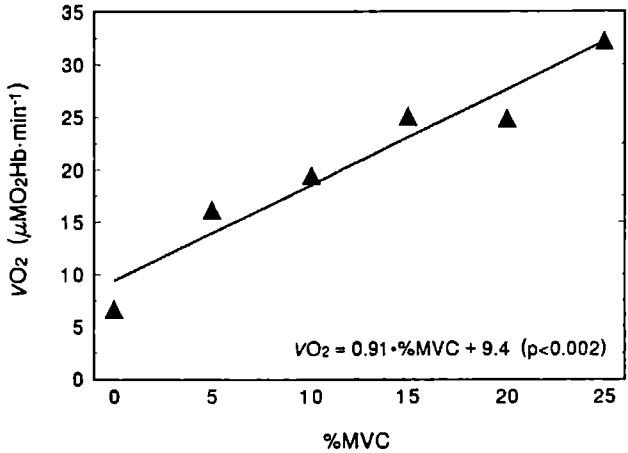

Figure 6.3 The relationship between oxygen consumption ($\dot{V}O_2$) and percentage of maximun voluntary contraction (%MVC) for all subjects (n=11). The values for the %MVC are averaged from the individual $\dot{V}O_2$'s of the subjects for that specific percentage MVC.

Discussion

In this study the possibility to assess $\dot{V}O_2$ non-invasively was demonstrated and the relationship between the relative isometric force level and $\dot{V}O_2$ was evaluated. The results proved to be reproducible. The inter-individual variation, however, was large. One of the factors which could play a role in this, is the inter-individual variation in pathlength factor. Ferrari *et al.* [1992] reported a pathlength factor variation between 3.8 and 5.1 in the forearm of 8 volunteers (inter-optode distance 3 cm). This variation does not fully explain the variation in $\dot{V}O_2$ in this study. More important factors might be the inter-individual differences in fat/muscle ratio and the relatively small soleus muscle. Both these factors imply that the amount of muscle tissue actively contributing to the NIRS signals can vary considerably.

Some subjects showed an increase in total blood volume during occlusion, both with and without isometric contraction. Since the cuff pressure was more then 270 mmHg it is unlikely that there was still an arterial inflow into the leg. A more reasonable explanation is a redistribution of the blood volume in the calf, due to gravitational forces. Another explanation might be volume changes caused by inflating the cuff which pushes blood to the distal part of the leg. This increase has also been observed by Cheattle *et al.* [1991], who used a similar set up.

The calculated value for $\dot{V}O_2$ at rest is in agreement with other studies. De Blasi *et al.* [1993], using NIRS, found a value for $\dot{V}O_2$ of 2.9 ± 0.4 μmol $O_2 \cdot min^{-1} \cdot 100\ g^{-1}$ in

the brachio-radial muscle of the forearm of 7 subjects. This is not significantly different from the result in this study ($p=0.44$). For comparison De Blasi's result was recalculated using a skeletal muscle density of 1.04 kg/l instead of the 1.33 kg/l used in their study. More recently Hartling *et al.* [1989] found a $\dot{V}O_2$ at rest of 6.1 ± 1.8 μmol $O_2 \cdot min^{-1} \cdot 100\ g^{-1}$ in the forearm of 5 volunteers. Although this value is higher than in the present study it is also not significantly different ($p=0.11$).

The significantly lower rest $\dot{V}O_2$ at the end of the experiment could be explained by the exercise prior to this measurement. A slightly higher muscle temperature would result in a higher muscle metabolism efficiency and therefore a lower $\dot{V}O_2$ [Sargeant *et al.* 1987].

For all subjects a linear relationship between force and $\dot{V}O_2$ was found. At a level of 45% of the maximum load Hartling *et al.* [1989] found a $\dot{V}O_2$ of 105 ± 10 μmol $O_2 \cdot min^{-1} \cdot 100\ g^{-1}$. Extrapolation of the graph in Figure 6.3 to an exercise level of 45% MVC leads to a $\dot{V}O_2$ of 20 $\mu mol O_2 \cdot min^{-1} \cdot 100 g^{-1}$, a considerably lower value. A straight forward comparison of both values however is not possible as in the study of Hartling dynamic exercise was performed while in the current study an extrapolated value for $\dot{V}O_2$ during isometric exercise was used. There are also considerable differences in the method of how the $\dot{V}O_2$ was assessed. The method of Hartling depends on the determination of several variables, like forearm volume, blood flow, and arterial and venous blood analysis. In this way more sources of error are introduced than using the NIRS technique, which has a more local and direct access to the investigated muscle.

Conclusion

NIRS appears to be a reproducible and reliable method for the non-invasive assessment of the $\dot{V}O_2$ in human muscles. Before the method can effectively be applied in, e.g., the evaluation of muscle training programs more research has to be done on the characterization of different muscle groups. The influence of muscle fiber type composition and fat/muscle ratio should be studied in more detail.

Acknowledgement

The authors wish to express their thanks to Mr. R. Houston for critical reading and to Mr. A.C.A. Vissers for valuable technical assistance.

Cerebral and circulatory haemodynamics before and during vasovagal syncope induced by orthostatic stress

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(Submitted)

Summary

Vasovagal syncope or common fainting is usually described as a sudden and transient loss of consciousness which resolves spontaneously Cardio-circulatory changes are well described during and before syncope. However, changes in the cerebral circulation are not well defined In this study near infrared spectroscopy (NIRS) was used to assess the cerebral circulation during 80° head-up (HU) tilt. To simulate central hypovolemia 500 ml of blood was drawn from each of 10 healthy subjects Cerebral oxygenation of the right frontal lobe was monitored using NIRS, a technique measuring concentration changes in oxy- and deoxyhaemoglobin (O_2Hb and HHb). Oxygenation Index (OI) was defined as the difference between O_2Hb and HHb. Blood pressure, heart rate and cardiac output were monitored using a finger plethysmographic device (Finapres). The protocol was divided into 2 stages, each consisting of a 15 min stabilisation period in supine (SUP) position, 15 min in HU position and again 10 min in SUP position. Between stage 1 and 2 blood was drawn from the subject at the Red Cross Blood Bank. Haemoglobin concentration (ctHb) was measured before and 30 min after withdrawal of blood No compensatory hemodilution was observed. During HU position in the second stage 6 subjects fainted (F), 4 did not (NF). A significant difference ($p=0.02$) between F and NF was found in the observation that prior to fainting the OI of F showed a steady decrease (-1.4 ± 0.5 μM/min) compared to NF (-0.18 ± 0.16 μM/min) This indicates that the onset of (pre)syncope is preceded by a mismatch between oxygen demand and oxygen supply in the cerebrum Using NIRS enabled us to monitor this mismatch and to predict the onset of a syncope *before* clear signs in cardio-circulatory variables were visible.

Introduction

Vasovagal syncope or common fainting is usually described as a sudden and transient loss of consciousness which subsides spontaneously [van Lieshout *et al.* 1991]. The cardiovascular changes that occur during vasovagal syncope are described by a number of studies [Asmussen *et al.* 1940, Bergenwald *et al.* 1977, Goldstein *et al.* 1982, Grubb *et al.* 1992]. They may be characterised as a sudden decrease in arterial pressure, heart rate and peripheral resistance. The syncope itself is usually preceded by a presyncope (faintness) with a variety of symptoms like nausea, dizziness, sweating and skin pallor. However, the mechanisms responsible for the onset of syncope are still not well defined.

Bergenwald *et al.* [1977] suggested that a low central blood volume, e.g. due to administration of vasodilating drugs, negative pressure to the lower body (LBNP) or spinal anaesthesia, is the main underlying cause of vasovagal syncope. Glaister *et al.* [1990], in a study using LBNP, stated that a reduction in cerebral oxygenation, detected by near infrared spectroscopy (NIRS), could be the trigger for the cardio-vascular decompensation seen as a response to LBNP. A similar finding was reported by Jørgensen *et al.* [1993] during sustained passive head-up tilt. They suggested that presyncopal signs developed because of a critically reduced cerebral blood flow (CBF), detected by transcranial Doppler sonography (TCD). In a head-up tilt

experiment to study patients with recurrent unexplained syncope Grubb *et al.* [1991], also using TCD, observed during LBNP a paradoxical vasoconstriction of the cerebrum. It is however not likely that these patients, with known absence of change in cerebral blood flow velocity during hyper- or hypocapnia, show normal physiological response to orthostatic stress. In healthy subjects Levine *et al.* [1994] found a cerebral vasoconstriction during LBNP detected by TCD. The magnitude however was small compared to the changes in systemic and forearm vascular resistance.

All of these investigations indicate the existence of a mismatch between oxygen demand and oxygen supply in the cerebrum during, but also before syncope. Using the TCD technique however has the disadvantage of only measuring blood cell velocity in the middle cerebral artery, which does not necessarily give information on cerebral tissue oxygenation. Using near infrared spectroscopy (NIRS), a direct and non-invasive method to assess cerebral tissue oxygenation, could overcome this problem. Glaister *et al.* [1990] used the NIRS technique, but did not quantify his findings in terms of absolute haemoglobin concentration changes. This makes the results difficult to interpret and to compare with other studies. In the current study NIRS was used as a *quantitative* technique to assess cerebral oxygenation and haemodynamics before and during vasovagal syncope. This is possible by incorporating a factor into the mathematical algorithm which accounts for the distance between the NIRS lightguides and the scattering of light in the tissue. Although the absolute level of the haemoglobin concentration in the brain can not be measured, the change in concentration can now be quantified. This makes the results easier to interpret and comparable with other studies.

Furthermore in this study the effect of a reduction in central blood volume was investigated, induced by drawing 500 ml of whole blood from the subject.

Material and methods

Subjects

Ten healthy, non-smoking male subjects (22-49 years, 171-199 cm, 61-110 kg) participated in the study. All subjects were normotensive and not on medication. All experiments took place in the morning. The subjects refrained from caffeine and alcohol at least one hour before the experiment, but were allowed to have a light breakfast. All subjects gave informed consent. The protocol was approved by the Ethics Committee of the University.

Instrumentation

The cerebral oxygenation was monitored using NIRS. The method has first been described by Jobsis [1977]. The technique is based on two fundamental characteristics: firstly the relative transparency of human tissue to light in the near infrared region and secondly the oxygenation dependent absorption changes in the cerebral tissue caused by chromophores, mainly oxy- and deoxyhaemoglobin (O_2Hb and HHb). By measuring changes in light absorption at different wavelengths tissue oxygenation can be monitored continuously. The relation between changes in absorption and in concentration are given by a modified Lambert-Beer law [Delpy *et al.* 1988], in which a pathlength factor is incorporated which accounts for the scattering of light in the tissue. In this study a pathlength factor of 6.0 was used [van der Zee *et al.* 1991]. The sum of O_2Hb and HHb, tHb is a measure for the total blood volume in the tissue. The oxygenation index (OI) was defined as O_2Hb-HHb. The inter-optode distance was 5.5 cm for all experiments. The NIRS equipment (Radiometer Medical A/S, Copenhagen, Denmark) used is a continuous wave, 4 wavelengths instrument. NIRS data were sampled every second, displayed real-time and stored on disk for off-line analysis. The algorithm used to convert absorption changes into concentration changes is described by Livera *et al.* [1991].

Blood pressure (BP) and heart rate (HR) were measured continuously with a plethysmographic device (Finapres, Ohmeda, USA). The instrument measures blood pressure in the finger and is based on the volume clamp method of Penaz [1973]. The cuff of the instrument is placed around the middle finger, which is held at the midaxilliary position at heart level. It has been shown that changes in intra-arterial blood pressure are accurately reflected by finger blood pressure measurements [Friedman *et al.* 1990, Imholz *et al.* 1990]. Data were collected on disk at a rate of 100 Hz for off-line calculation of stroke volume (SV) using the pulse-contour method [Wesseling *et al.* 1983] and, by multiplication with HR, of cardiac output (CO). Without individual calibration absolute values for SV and CO can not be obtained, changes however can accurately be monitored.

The arterial saturation (SaO_2) was monitored with a pulse oximeter (N200, Nellcor, USA). A blood sample was taken in sitting position by venepuncture. The total haemoglobin concentration of the sample (ctHb) was measured with an automated cell counter (Coulter JT3, Coulter Electronics Ltd, UK)

Protocol

The experiment was divided into 2 stages. The first stage of the experiment took place at the Physiological Laboratory of the department. The ambient temperature of the Laboratory was kept at a constant level of 21°C. The optodes of the NIRS were attached on the left side of the forehead. The cuff of the Finapress was attached

to the middle finger of the right hand. The right arm was supported so that during vertical posture the hand could be held passively at heart level. The probe of the pulse oximeter was attached to the index finger of the left hand. To prevent falling during syncope the subjects were fixed onto the tilt table using straps. The head-up tilt was practised twice before the start of the experiment. The experiment started with a 15 min stabilisation period in supine position. After this period a 80° passive head-up tilt was performed. The head-up tilt was maintained for 15 min or until the subjects developed signs of presyncope. The subject was then immediately returned to supine position. Measurements went on for an additional 10 min during the recovery period.

Directly after the first stage 500 ml of whole blood was drawn from the subject. This was done in the laboratory of the Red Cross Blood Transfusion Services. The standard procedures for the withdrawal of blood were followed. The blood drawn from the subjects was used for regular purposes. ctHb was measured just before the withdrawal of blood and 30 min after withdrawal.

After the withdrawal of blood the subjects came back to the Physiological Laboratory for the second stage of the experiment, starting approximately 45 min later. The protocol was the same as during the first stage.

Analysis

In the supine position baseline values (SUP) for the NIRS, BP, HR, CO and SV signals were taken, being a 2 min average prior to the head-up tilt. The signals were analysed once more using a 30 sec average in head-up position (HU), 1 min after postural circulatory adjustment. For the NIRS signals the changes in concentration from baseline values to head-up values were taken, for the other variables the absolute signals were used. The start of presyncope was defined as the time were systolic and diastolic blood pressure started to show a constant decrease immediately before the subject was returned to supine position while not completing the 15 min head-up tilt. On the basis of the occurrence of presyncopal signs during the head-up tilt of the second stage of the experiment the subjects were divided into 2 groups: fainters (group F) and non-fainters (group NF). As a measure for mismatch in oxygenation the time course of the OI during de head-up tilt was taken at both stages of the experiment. Therefore the slope of the regression line of the OI was calculated. For group F also the slope of the OI during presyncope was calculated. Figure 7.1 shows a schematic presentation of the signal analysis.

To compare ctHb before and after withdrawal of blood a paired t-test was used. To compare before withdrawal and after withdrawal as well as base line values with head-up values a nonparametric t-test was used. Results between group F and NF

were compared using the Wilcoxon 2-sample test. The slopes of the OI were calculated using linear regression analysis. Results are given as mean ± S.D.

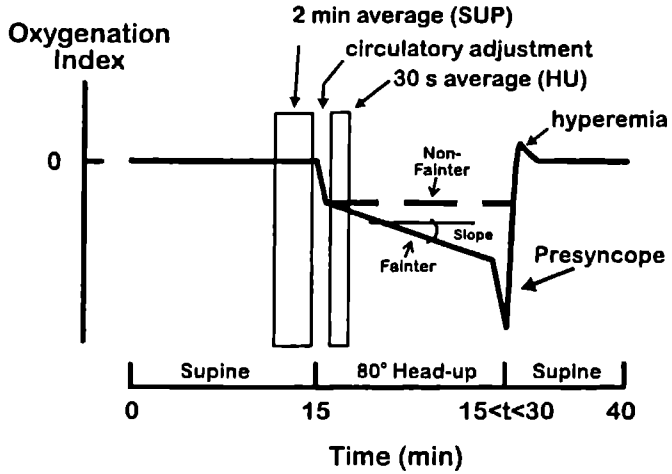

Figure 7.1 A schematic representation of the time course of the experiment and the analysis performed on the signals. After 15 min in supine (SUP) position the subject is tilted to an 80° head-up position (HU) for 15 min , or less if the subject faints. The experiment ends with a 10 period in supine position. For clarity only the oxygenation index (OI), being the difference of oxy- and deoxyhaemoglobin, is displayed.

Results

The ctHb after the withdrawal of blood was not significantly different compared to ctHb before the donation (both 9.3 ± 0.4 mM). In Table 7.1 the results for the NIRS signals and SaO_2 are given. None of these changes were significant when comparing before and after withdrawal of blood or from the SUP to HU position. During all experiments SaO_2 was never lower then 96%. The cardio-circulatory signals are given in Table 7.2. In both stages of the experiment there was a significant difference between the SUP and HU position, except for systolic BP. The only significant difference comparing equivalent positions before and after donation was in the HR in the HU position.

Signs of presyncope occurred in 6 subjects (group F) in the HU position during the second stage of the experiment after an average of 7.6 (range: 3-13) min. The other 4 subjects (group NF) showed no signs of presyncope during the 15 min head-up tilt.

Table 7.1 The change (Δ) in NIRS signals and arterial saturation (SaO_2) from supine position (SUP) to head up position (HU) before and after withdrawal of blood (n=10) No significant differences were found (SUP vs HU nor Before vs After)

	Before withdrawal (SUP → HU)	*After withdrawal* (SUP → HU)
ΔO_2Hb	0 6 ± 4 3	-0 5 ± 2 6
ΔHHb	2 2 ± 2 4	2 5 ± 2 0
ΔtHb	2 8 ± 4 3	2 0 ± 3 1
ΔOI	-1 6 ± 5 4	-3 0 ± 3 5
ΔSaO_2(%)	0 4 ± 0 7	1 8 ± 2 1

Units NIRS μM (mean ± S D)

In all situations and for all signals group F was compared with group NF. Significant differences were found only in the case of the HR after donation in as well the SUP (F: HR= 75 ± 5 min^{-1}, NF: HR= 60 ± 5 min^{-1}, $p<0.01$) and HU (F: HR=121 ± 8 min^{-1}, NF: HR= 86 ± 11 min^{-1}, $p<0.01$) position. The most striking difference was found in the rate of deoxygenation (time course of OI), were group F had a significant higher rate than group NF after withdrawal of blood (Table 7.3).

Table 7 2 The cardio-circulatory variables before and after withdrawal of blood in both supine (SUP) and head up (HU) position (n=10) The only significant difference between fainters and non-fainters was found after donation in the HR, in both SUP and HU position See text for more details

	Before withdrawal		*After withdrawal*	
	SUP	HU	SUP	HU
DIA BP (mmHg)	67 ± 9	86 ± 10**	66 ± 12	84 ± 16**
SYS BP (mmHg)	134 ± 11	145 ± 12	133 ± 17	138 ± 19
HR (min^{-1})	66 ± 7	89 ± 13**	69 ± 9	107 ± 20** ^
SV (mL min^{-1})	77 ± 8	49 ± 12**	72 ± 12	41 ± 12**
CO (L min^{-1})	5 1 ± 0 8	4 3 ± 0 8**	4 9 ± 1 1	4 3 ± 1 3*

* HU position significant different compared to SUP position ($p<0.05$)

** HU position significant different compared to SUP position ($p<0.01$)

^ HU position after donation significant different compared to HU position before donation ($p<0.01$)

Table 7.3 The rate of change (slope) of oxygenation index (OI) and blood volume (tHb) in the head-up position, before and after withdrawal of blood After withdrawal a significant difference (*p=0 02) in OI is found between the fainters (n=6) and the non-fainters (n=4) No significant differences were found for the tHb signal

	Before withdrawal		*After withdrawal*	
	Fainter	Non-Fainter	Fainter	Non-Fainter
Slope tHb	0 15 ± 0 13	0 08 ± 0 08	0 03 ± 0 45	0 19 ± 0 05
Slope OI	-0 06 ± 0 18	-0 16 ± 0 31	-1 4* ± 1 4	-0 18 ± 0 33
Slope OI (during presyncope)	N A	N A	-5 2* ± 4 2	N A.

Units μM min^{-1} (mean ± SD), N A not applicable

Discussion

From the ctHb values before and after withdrawal of blood it can be concluded that the central blood volume was decreased by the donated volume (500ml), being approximately 10% of the blood volume of the body. No significant haemodilution was found within 30 min after the donation. This fall in circulating blood volume was not compensated for by cardio-circulatory changes (Table 7.2), except for an elevated HR in HU position. This means that the subjects experienced a normotensive hypovolemia. None of the changes in cardio-circulatory variables from the SUP position to HU position differed from the findings reported by others. In this study too it was found that group F had a significant higher HR in the HU position compared to group NF [Bergenwald *et al.* 1977, Harkel *et al.* 1993]. Also in the SUP position group F had a significant higher HR. Although it seems that this higher HR indicates a predictive value for syncope [Graham 1961, Bergenwald *et al.* 1977] one has to bear in mind that the HR variability of a total population will be higher than in the (relatively) young, healthy subjects of this study.

In group F a constant decrease of the oxygenation index in HU position after the withdrawal of blood was observed, which was not found in group NF or before donation. This can not due to a change in SaO_2, which was never lower then 96% in any of the subjects. During presyncope the OI even decreased by almost a factor four. This is shown in Figure 7.2, where the tracings the NIRS signals as well as BP signals of a fainter before and after withdrawal of blood are given. For comparison the tracings of a non-fainter are given in Figure 7.3.

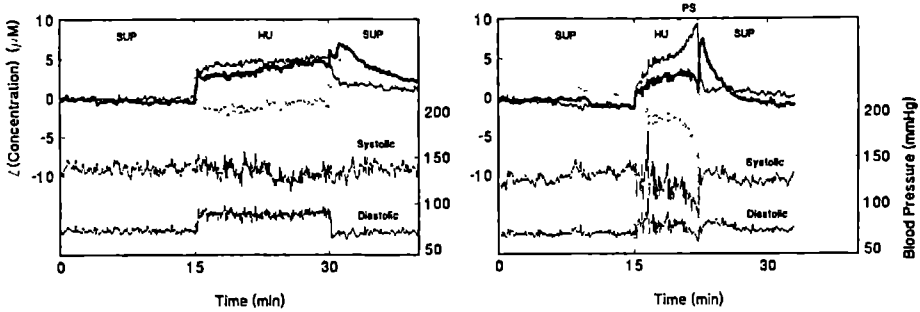

Figure 7.2 In these panels the oxyhaemoglobin (O_2Hb: ·····), deoxyhaemoglobin (HHb: ——), total haemoglobin (tHb:▬▬) and systolic and diastolic blood pressure signal of a fainter are displayed. In the left panel, before withdrawal of blood, stable signals in both supine (SUP) and head-up (HU) position can be observed. This is no more the case after withdrawal of blood, seen in the right panel. The subjects shows signs of presyncope after approximately 5 min in supine position.

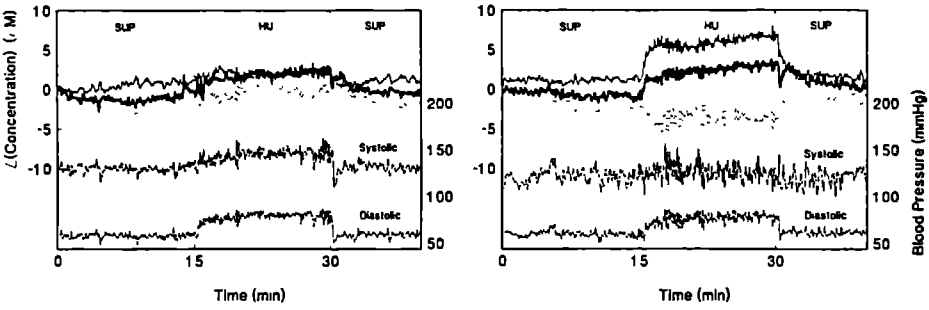

Figure 7.3 The two panels show the tracings of oxyhaemoglobin (O_2Hb: ·····), deoxyhaemoglobin (HHb: ——), total haemoglobin (tHb:▬▬) and systolic and diastolic blood pressure signals of a non-fainter. The left panel is before, the right panel after withdrawal of blood. Although the changes of the NIRS signals are greater after withdrawal of blood, they stay stable in the head-up position after a short stabilisation period. The variation in blood pressure in both supine (SUP) and head-up (HU) position is greater then before withdrawal of blood.

After some experience the experimentors were able to forecast the onset of (pre)syncope on the basis of the OI only and before any other clear signals were available. So a constant decrease of the OI seems to be a reliable early warning indicator for presyncope. This suggests that the cerebral circulation in group F during the HU position is insufficient. Assuming an unchanged cerebral oxygen consumption after tilting the decrease of the OI suggest a corresponding decrease in CBF, resulting later in presyncope with concomitant fall in BP. This is also illustrated in Figure 7.4, where NIRS and HR signals of a pilot experiment for the

current study are given. In this experiment a 29 year old female was tilted twice. No blood withdrawal took place. The first 80° head-up tilt (HU1) lasted 3 min. Between the first and second tilt there was a 3 min period in supine position. During the second tilt (HU2) a same pattern in the NIRS signals as during the first tilt was observed. There was an instant decrease in tHb of 3.9 μM directly after both head-up tilts. After 5.8 min in head-up position during the second tilt the subject showed signs of presyncope and was brought back in supine position. From the O_2Hb and HHb it is now clear that the syncope could have been predicted during the first head-up tilt.

An indication for this decrease in CBF before the onset of presyncope has also been found by Jørgensen *et al.* [1993] using TCD. This was concluded from the finding that before presyncope the middle cerebral artery mean velocity decreased in a head-up tilt experiment with healthy subjects. Another change in circulatory variables prior to presyncope was found by Harkel *et al.* [1993], who showed that in healthy subjects during prolonged standing fainters showed a gradual decrease in systemic vascular resistance, not found in the non-fainters.

The results of the other NIRS signals (Table 7.1) illustrate the high inter-individual variability. However, the individual tracings showed well-defined differences during change of posture (Figure 7.2 and Figure 7.3). The constancy of the cerebral blood volume (tHb) during HU position in group F indicate that the mismatch between oxygen supply and demand is not compensated for by vasodilatation in the small vessels, as could be expected on basis of the autoregulatory mechanism [Mchedlishvili 1986]. During presyncope itself also no significant vasodilatation was found. This is in conflict with the study of Grubb *et al.* [1991], using TCD, who found a paradoxical vasoconstriction prior to syncope. This was however in a patient group, where the regulation of cerebral blood flow might be different from that in healthy subjects. A similar finding was reported by Levine *et al.* [1994], using TCD, now in healthy subjects. In the study graded orthostatic stress was induced using lower body negative pressure. The arteriolar vasoconstriction reported however was small. The changes in peripheral resistance were found to be much greater and of more importance. A limitation of NIRS in this is that it is not exactly known which vascular compartment has the major contribution to the signals. It might well be that a (small) vasoconstriction in the arteriolar vessels of the cerebrum can not be detected by NIRS.

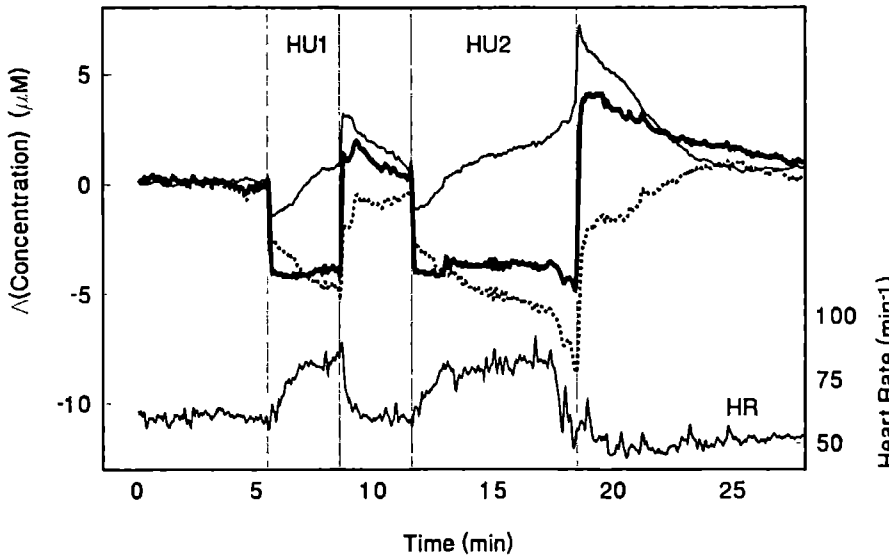

Figure 7.4 The oxyhaemoglobin (O_2Hb: ·····), deoxyhaemoglobin (HHb: ——), total haemoglobin (tHb: ▬▬) and heart rate (HR) signals of a pilot experiment, in which a 29 year old female was tilted twice. No blood withdrawal took place. The first 80° head-up tilt (HU1) lasted 3 min. Between the first and second tilt there was a 3 min period in supine position. During the second tilt (HU2) a same pattern in the NIRS signals as during the first tilt was observed. There was a high and immediate decrease in tHb of 3.9 μM directly after both head-up tilts. After 5.8 min in head-up position during the second tilt the subject showed signs of presyncope and was brought back in supine position before complete collapse. From the O_2Hb and HHb signals it is now clear that the syncope could have been predicted during the first head-up tilt.

Conclusion

It is concluded that the onset of vasovagal (pre)syncope is preceded by a mismatch between oxygen demand and oxygen supply in the cerebrum. Using NIRS enables us to monitor this mismatch on a safe and continuous basis and to predict the onset of a syncope *before* clear signs in cardio-circulatory variables are visible. This study also stresses the fact that the description of syncope as a sudden onset phenomenon has to be revised.

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Epilogue

Ever since the introduction of NIRS by Jöbsis in 1977 the technique is regarded to be promising with a high potential. It was and is the only easy applicable technique giving real-time information on the oxygenation state (deep) within various types of tissue. Fact however is that NIRS is still not a widely used method. This can partly be explained from the fact that the technology needed was not available. At the moment this is no more the case. High power laser diodes e.g. can be obtained at reasonable prices, even though the market is partly shielded by some companies. A more serious problem is the lack of quantitation. This makes NIRS first of all a trend monitor. Only in combination with other techniques, like pulse oximetry, it is possible to obtain absolute values for tissue blood volume and flow. By applying these methods however NIRS looses part of its charm. To become an established method the technique of NIRS has to be refined and extended. Some of the problems which have to be solved in the years coming are discussed in the next paragraphs.

Multi-side detection
In many situations it could be helpful to have information from more than one side of on organ or limb. These situations include carotid surgery, brain surgery and situations were comparison with a control limb or organ is of importance, e.g. muscle ischaemia-reperfusion experiments. Multi-side detection with minimal invasive optodes could be a valuable tool for local oxygenation monitoring in compromised organs, e.g. liver or kidney, during intensive car. Technically this extension should be no problem.

Spectral extinction coefficients
No consensus exists about the best possible data set for the various chromophores. This results in small differences, affecting the accuracy of the system. The influence on the algorithm of other derivatives of haemoglobin, like carboxy- and methaemoglobin, should be studied. If there is an effect, these chromophores should be incorporated into the algorithm to raise the accuracy of quantitation. In case of muscle tissue the effect of myoglobin on the haemoglobin signal has to be investigated.

The pathlength factor
A great step forwards to quantitation was the introduction of the "differential pathlength factor". It is now possible to quantitate *changes* in haemoglobin concentration. Unfortunately the pathlength factor is only known for a limited number of tissue samples. Testicular tissue is for example not on the list. With most NIRS instruments it is not possible to measure the pathlength factor and therefore correction for inter-individual differences is impossible. Direct measurement of the

pathlength factor by phase-resolved spectroscopy can overcome this problem. Technically this extension is possible.

Standardisation
To become an accepted method it should be possible to compare results world-wide. A minimum demand is the presentation of results in accepted units. When using derived methods, like measurement of tissue blood volume or flow, standardised protocols should be used. For some manufactures it means that they should give access to the implemented algorithm as well as to the raw data.

Simplicity
Most of the current available instrumentation is, although simple in nature, not user friendly. Partly this is due to fact that absolute quantitation is not yet possible. The ideal situation would be an instrument with a front panel only consisting of a display giving "tissue saturation" and indicators giving information on the patient's condition and about the reliability of the signals.

Another cumbersome matter is the development of easy to use optodes for various clinical situations. Gluing the optodes onto the patients head and wrapping it in black clothes can not be considered elegant clinical practise.

What are we looking at?
It is known that NIRS measures deep within tissue. Which vascular compartments are monitored is not known. Large vessels will only partly contribute to the detected signal as most photons will be absorbed in these vessels. For the interpretation of the results however it is important to know what vascular compartment(s) contribute to the detected concentration changes.

The first question has yet to be answered: "Is Near Infrared Spectroscopy a toy or a tool?". Looking at the contents of this thesis it is justified to conclude that NIRS is a valuable extension of currently available research tools. Even if absolute quantitation is not within reach before the next decade, NIRS can be a valuable addition to the monitoring equipment currently present at the Intensive Care Unit or in the operating theatre, where, e.g., brain injuries are common complications of surgery during cardio pulmonary bypass (5-10%). A new field will also be the exploration of NIRS in muscle physiology and pathophysiology.

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Summary

In this thesis the results of several studies are described having Near Infrared Spectroscopy (NIRS) as the common factor. NIRS is a continuous, non-invasive and direct way to assess tissue oxygenation and haemodynamics, based on the existence of chromophores with a light absorption dependent on the available oxygen. Emphasis is not given to the widespread application of NIRS in the field of Neonatology, but to the exploration of new fields. This has resulted in studies on adult cerebral circulation, oxygen consumption in human skeletal muscle and the blood supply of testicular tissue.

Chapter 1 starts with a short introduction on oximetry in general, followed by an outline of NIRS. The principles of NIRS are described, with main attention for the "continuous wave" technique, used in our Laboratory. The influence of the spectral extinction coefficients and the pathlength factor on the results are described. The combination of NIRS with pulse oximetry provides the possibility to determine absolute tissue blood volume and flow. The chapter finishes with the aim of the thesis and a brief introduction to the following chapters.

In Chapter 2 the algorithm used in NIRS is evaluated. With the algorithm is meant the set of linear equations resulting from the Lambert-Beer law of a multi-component system. If the same wavelengths are used the algorithm will be independent of the instrument. The reality however is that different research groups use different algorithms, which can not always be explained by differences in wavelengths of the light sources. In this chapter therefore the influence of (slightly) different algorithms is investigated in an *in vitro* system. In the system whole blood (scattering) or a stroma-free haemoglobin solution (non-scattering) was circulating. The oxygen saturation could be varied. It shows that absolute quantitation with the current technology is not possible, but that there is a linear relationship between saturation changes and concentration changes over the whole saturation range. It was concluded that two of the most widespread algorithms show similar behaviour.

The "continuous wave" type instrument like the one used in the Nijmegen Laboratory is regarded to be the gold standard in NIRS. A problem is the quantitation of the signal. For daily clinical practise a numerical displayed value of, e.g., regional tissue saturation is invaluable. This has been realised in the INVOS 3100 of Somanetics Inc., USA, measuring regional cerebral tissue saturation (rSO_2). The rSO_2 should reflect mainly venous saturation. The instrument makes use of two separate detectors. The difference signal of the two detectors should correct for the contribution of the skin and skull to the cerebral oxygenation. In Chapter 3 this

INVOS instrument is compared to a conventional NIRS instrument. Three types of intervention were used in a group of 18 healthy volunteers. The first was a small and transient decrease of inspired oxygen concentration. Assuming an unchanged cerebral blood flow, both arterial saturation and rSO_2 should decrease. The other interventions were hyper- and hypocapnia. In this case no significant change in arterial saturation is to be expected, but due to the change of the carbon dioxide level in the blood the cerebral blood flow will either increase (hypercapnia) or decrease (hypocapnia). This was monitored with both instruments. Assuming a constant oxygen consumption of the brain, this should result for the INVOS in either an increased (hypercapnia) or decreased (hypocapnia) rSO_2. A reasonable correlation between the conventional NIRS instrument and the INVOS instrument was found during hypercapnia. The INVOS instrument was not sensitive enough to detect changes in rSO_2 during hypocapnia, whereas the gold standard NIRS instrument could. The variation in the rSO_2 in rest was high (range 59-79%). No correlation was found with any of the other signals. It was concluded that the INVOS correlated with the conventional NIRS, but that the absolute rSO_2 reading does not reflect reality and is misleading. Furthermore, the INVOS signal averaging algorithm is to slow and needs improvement. For these reasons the INVOS instrument is merely a trend monitor in situations were large changes in cerebral oxygenation are to be expected.

Cryptorchidism is a congenital defect characterised by a failure of the testis to descend into the scrotum. It is the most common male sexual disorder with an incident of about 1% in one year old boys. In case of a surgical procedure (orchiopexy) the urologist has the choice of two options. The first is autotransplantation, which gives good result but is difficult, painful and time consuming. The second is a two-stage orchiopexy, which can even be done laparoscopically in day-care. The results however are not always predictable. There is however no objective criterion to choose between the two types of surgery. In Chapter 4 an animal model is developed in which reproducible results of the measurement of the ATBV in abdominal testes were obtained by combining NIRS with pulse oximetry testis. In Chapter 5 this method is applied during a two stage orchiopexy. During the first operation the ATBV was measured and on the basis of this measurement a prediction was made regarding the long-term viability of the testis. The second operation was approximately 2½ months later. It was found that testis viability was correctly predicted during the first operation. It is concluded that this method might also be applied to other types of (pedicled) surgery where the examination of tissue viability is of the utmost importance.

In Chapter 6 NIRS is applied on human skeletal muscle. This study is one of the first to actually quantify the regional oxygen consumption of a muscle non-invasively. The soleus muscle was studied during graded isometric exercise and during rest. The exercise levels ranged from 5% to 25% of the maximum voluntary contraction. The oxygen consumption was derived from the decrease in oxyhaemoglobin directly after a complete arterial occlusion be means of blood pressure cuff around the leg. In all cases a linear relationship was found between the exercise level and the oxygen consumption. The oxygen consumption found at rest was comparable with other studies, using invasive techniques. The inter-individual variation among subjects was high. Possible reasons for this might be the relative smallness of the soleus muscle, differences in fat percentage and differences in the delivered maximun voluntary contraction. It is believed that NIRS applied to skeletal muscle can add valuable information to current available knowledge. It is possible to study relatively easy regional differences in oxygen consumption in a muscle or muscle groups. It should be possible to follow the effect of training programme in time.

Vasovagal syncope can be described as a sudden and transient loss of consciousness with subsequent loss of vertical posture. The syncope itself is preceded by a presyncope, characterised by dizziness, nausea and skin pallor. Prolonged standing, but also loss of central blood volume can result in syncope in healthy humans. The behaviour of cardio-circulatory variables like blood pressure, heart rate and peripheral resistance are well described. Only little however is know about the changes in cerebral circulation. In Chapter 7 NIRS was used to study the adult cerebral circulation during orthostatic stress, induced by a combination of 80° head-up tilt and withdrawal of 500 mL of whole blood. The donation of blood took place at Red Cross Blood Bank. The blood was used for regular purposes. Ten healthy male volunteers participated in the study. It was found that 6 of the 10 volunteers fainted within a 15 min head-up tilt period after withdrawal of blood. Cardio-circulatory variables like blood pressure and heart rate showed results similar to earlier studies. On the basis of these variables fainting could not be foreseen. The cerebral circulation between the fainters and the non-fainters showed a significant difference in the observation that the oxygenation index (difference between oxy- and deoxyhaemoglobin) of the fainters showed a constant decrease from the moment the fainters were placed in the head-up tilt position. This was not seen in the non-fainters. Assuming that the cerebral oxygen consumption does not change after tilting a subject from supine to head-up position this indicates a decrease in cerebral

blood flow. It was concluded that (pre)syncope is preceded by a mismatch between oxygen demand and oxygen supply in the cerebrum. Using NIRS enables us to monitor this mismatch and to predict the onset of a syncope *before* clear signs in cardio-circulatory variables are visible.

The thesis ends with an Epilogue in which a view on the future of NIRS is given. Although NIRS has proven to be a reliable method, undoubtly adding value to various types of research, the method has to adapt and extent in the coming years. Only then can NIRS develop into an established method.

Samenvatting

In de lucht die wij dagelijks inademen bevindt zich ongeveer één vijfde deel zuurstof. In de longen wordt die zuurstof gebonden aan het bloed en naar de rest van het lichaam getransporteerd. Het dagelijkse zuurstofverbruik van ons lichaam bedraagt vele liters. In het algemeen zijn wij ons hier niet van bewust. Dat gebeurt pas op het moment dat zuurstof niet meer of niet in voldoende mate aanwezig is (grote hoogten of verdrinking) of als het lichaam niet meer in staat is voldoende zuurstof op te nemen (aandoeningen van long en luchtwegen). Het kan ook zijn dat het grootste deel van het lichaam wel voldoende zuurstof krijgt en het tekort zich beperkt tot een orgaan of ledemaat. Denk hierbij bijvoorbeeld aan "etalage benen" waarbij mensen ten gevolge van een slechte doorbloeding van het been niet meer in staat zijn grotere stukken achter elkaar te lopen. Het nemen van een pauze, bijvoorbeeld voor een winkel etalage, wordt essentieel. Een andere situatie doet zich voor in de operatiekamer van een ziekenhuis. Tijdens open hartoperaties wordt de beademing en de hartfunctie door machines overgenomen. Tevens wordt de temperatuur van de patiënt verlaagd. De zuurstofvoorziening (oxygenatie) van de hersenen kan door deze ingrepen in het gedrang komen, met alle schadelijke gevolgen van dien.

Het is dan ook van belang om over een techniek te beschikken waarmee de oxygenatie toestand van hersenweefsel, maar ook van spier of ander weefsel onderzocht kan worden. Dit proefschrift gaat in op de (klinische) toepassing van een dergelijke methode: nabije infrarood spectroscopie, kortweg NIRS. Dit is een relatief nieuwe methode die een aantal grote voordelen heeft: niet invasief, m.a.w. er hoeft niet geprikt, geslikt of gesneden te worden, de meetgegevens worden direct weergegeven op een computerscherm en de techniek heeft een hoge tijdresolutie.

In de beginjaren van NIRS, maar ook nog heden ten dagen, werd de techniek voornamelijk toegepast bij te vroeg geboren baby's. De techniek heeft echter een veel groter potentieel. De doelstelling van het onderzoek dat in dit proefschrift is beschreven was dan ook om dit potentieel te onderzoeken.

NIRS werkt met licht in het verre (zichtbare) rood en infrarood. In dit gedeelte van het spectrum kan het infrarode licht relatief gemakkelijk in de weefsels doordringen. Het doorstralen van 6 tot 9 centimeters weefsel, bijvoorbeeld een babyhoofdje of de onderarm, is mogelijk. Het licht wordt in het weefsel gedeeltelijk verstrooid en gedeeltelijk geabsorbeerd. Al naar gelang er meer of minder zuurstof aanwezig is in het bloed zal het van kleur veranderen. Denk hierbij aan het "blauw zien" van mensen die in ademnood verkeren. De mate van kleurverandering beïnvloedt weer de intensiteit van het licht dat door het weefsel gestraald wordt. De verhouding van intensiteit van het ingestraalde licht en het opgevangen licht is een maat voor de doorbloeding. Het molecuul dat verantwoordelijk is voor de

kleurverandering is het hemoglobine, waaraan al dan niet zuurstof gebonden kan zijn (respectievelijk oxy- en deoxyhemoglobine).

In Hoofdstuk 1 en 2 wordt de precieze werking van NIRS uitgelegd en wordt de combinatie met puls oximetrie beschreven. Met behulp van puls oximetrie is het mogelijk niet invasief de slagaderlijke zuurstofverzadiging te bepalen. Combinatie van beide methoden maakt het mogelijk het absolute bloedvolume en de bloedstroomsterkte van een orgaan te bepalen. Ook wordt ingegaan op het effect van kleine veranderingen in het rekenmodel, dat veranderingen in lichtabsorptie omzet in concentratie veranderingen, op het eindresultaat.

In Hoofdstuk 3 wordt het in Nijmegen gebruikte conventionele NIRS instrument, dat als "gouden standaard" gezien wordt, vergeleken met een ander instrument, de INVOS 3100 van de firma Somanetics. De INVOS rekent concentratie veranderingen om naar een absolute waarde van de zuurstofverzadiging van het hersenweefsel. De toepasbaarheid in de kliniek zou hiermee op grotere schaal mogelijk worden: een getal is immers gemakkelijker en sneller te interpreteren dan een aantal curven op een beeldscherm. Om de INVOS te testen werden bij gezonde vrijwilligers een aantal experimenten uitgevoerd waarbij de hersendoorbloeding en de zuurstofverzadiging van het bloed veranderd werden. Als eerste werd er minder zuurstof aan de proefpersoon aangeboden. Verwacht werd dat dit zou resulteren in een daling van zowel de slagaderlijke zuurstofverzadiging als van de hersenoxygenatie. Vervolgens werd het kooldioxyde gehalte in het bloed verhoogd respectievelijk verlaagd. In beide gevallen werd verwacht dat de slagaderlijke zuurstofverzadiging gelijk zou blijven maar de hersenoxygenatie zou stijgen respectievelijk dalen door de aanwezigheid van meer (hypercapnie) of minder (hypocapnie) kooldioxyde in het bloed. In alle gevallen bleken de veronderstellingen te kloppen en kon dit met zowel het conventionele NIRS instrument als met INVOS aangetoond worden. Wel bleek dat de gevoeligheid van de INVOS te gering was om het effect van hypocapnie aan te kunnen tonen. Verder bleek dat de rekenkundige middelingsprocedure van de INVOS te traag is om snelle veranderingen in het signaal te kunnen volgen. Het sterke punt van de INVOS, het in getal en maat uitdrukken van de hersenoxygenatie, bleek niet te voldoen. De gevonden resultaten in de rustwaarde liepen uiteen van 59% tot 79%. Dit is een te groot bereik om nog acceptabel te zijn. Concluderend kan gesteld worden dat de INVOS voldoet als (langzame) trendmonitor in situaties waar men grote veranderingen verwacht.

In Hoofstuk 4 en 5 wordt een urologisch onderzoek beschreven. Cryptorchisme, ofwel een niet in de balzak ingedaalde testikel, is een aangeboren afwijking die bij ongeveer 1% van de één jaar oude jongetjes voorkomt. Indien er chirurgisch ingegrepen moet worden (orchiopexy) heeft de uroloog de keus uit een aantal opties. De meest succesvolle, maar ook pijnlijkste en moeilijkste optie is autotransplantantie, waarbij de testikel losgeprepareerd wordt uit de buikholte en in de balzak wordt geplaatst. Een andere en veel minder ingrijpende optie is een orchiopexy in twee fasen, waar enige maanden tussen liggen. Een klein gaatje in de buikwand (laparoscopie) is voldoende om de ingreep, die veel minder belastend is voor de patiënt dan een autotransplantatie, uit te voeren. De uitkomst van deze ingreep is echter moeilijk te voorspellen. Het ontbreekt namelijk aan een betrouwbare test waarmee een keus tussen de verschillende opties gemaakt kan worden.

In Hoofdstuk 4 wordt een experiment beschreven dat het mogelijk maakt om het nog circulerende bloed in een testikel te meten nadat de hoofd bloedvoorziening (de vasa spermatica) is afgesneden zoals dat tijdens een orchiopexy in twee fasen ook gebeurt. Aan de hand van de resultaten van dit onderzoek wordt in Hoofdstuk 5 een voorspelling gedaan over de levensvatbaarheid van een testikel nadat de vasa spermatica is afgesneden. Na 2½ maand wordt de testikel weer onderzocht en indien geen afsterving heeft plaatsgevonden wordt het circulerende bloedvolume opnieuw bepaald. De gedane voorspellingen bleken met de praktijk overeen te stemmen. Deze methode zou ook goed toegepast kunnen worden bij transplantaties van andere organen waarbij het beoordelen van de levensvatbaarheid van het transplantaat van groot belang is.

In Hoofdstuk 6 wordt NIRS toegepast om de oxygenatie van menselijke skeletspieren te bepalen. Dit is de eerste studie die op een niet invasieve manier de zuurstof opname tijdens inspanning bepaalt.

De zuurstof opname van de musculus soleus, een spier in de kuit, wordt bepaalt. Dit gebeurt zowel in rust als tijdens isometrische inspanning, waarbij de kracht varieert van 5% tot 25% van de maximaal vrijwillige kracht. In alle gevallen werd een lineair verband gevonden tussen zuurstof opname en de geleverde kracht. Wel was de spreiding in de resultaten erg groot. De mogelijkheid om NIRS toe te passen bij spierfysiologisch en pathofysiologisch onderzoek opent mogelijkheden voor nieuwe onderzoekslijnen.

Flauwvallen (syncope) is een verschijnsel waar iedereen wel eens direct of indirect mee te maken heeft gehad. Is de oorzaak van het flauwvallen gelegen in

bijvoorbeeld lang staan (soldaat op wacht) of warmte ("door de warmte bevangen worden") dan kan men spreken van een vasovagale syncope. Tijdens het flauwvallen vinden er vrij plotseling een groot aantal veranderingen in het lichaam plaats waardoor men niet in staat is te blijven staan. Het belangrijkste is de plotselinge daling van de bloeddruk, vaak in combinatie met een daling van de hartslag. Deze reacties zijn allen beschreven in de literatuur. Minder duidelijk is wat er met de hersendoorbloeding gebeurt, zowel voor als tijdens syncope. In Hoofdstuk 7 wordt de hersendoorbloeding dan ook nader bestudeerd met behulp van NIRS tijdens een "til-tafel" experiment. Behalve de hersendoorbloeding werden ook de bloeddruk, hart minuut volume en de slagaderlijke zuurstofsaturatie continu geregistreerd.

Voor het experiment namen 10 gezonde vrijwilligers plaats op een bed dat na een aantal minuten gekanteld werd. Hierdoor werd de proefpersoon op een passieve manier gedurende 15 minuten rechtop gezet. Het bloed "zakt naar de benen". Omdat het hart per slag minder bloed kan pompen zal de hartslag toe nemen om het hart minuut volume gelijk proberen te houden. Na afloop van de eerste fase van het experiment werd er een halve liter bloed gedoneerd bij de Bloedbank van het Rode Kruis in Nijmegen. Binnen 30 minuten werd het experiment nogmaals uitgevoerd. Het bleek dat tijdens het eerste experiment niemand flauw viel. Tijdens het tweede experiment vielen 6 van de 10 proefpersonen flauw na gemiddeld 7½ minuut te hebben gestaan. De bloeddruk reacties en hartslag veranderingen waren gelijk aan wat al eerder in de literatuur beschreven is. Nieuw was de waarneming dat bij de groep flauwvallers er een constante daling in hersenoxygenatie werd waargenomen die begon vanaf het moment dat de proefpersoon rechtop gezet werd. Hieruit kon geconcludeerd worden dat de bloedstroomsterkte naar de hersenen te gering was. Dit was niet het geval bij de groep die niet flauwviel. Het bleek dat aan de hand van die daling van de hersenoxygenatie het flauwvallen voorspeld kon worden. De conclusie van dit onderzoek is dat de vasovagale syncope niet het plotselinge opzettende fenomeen is zoals vaak beschreven maar dat hieraan voorafgaand in ieder geval een verlaging van hersendoorbloeding optreedt.

Dit proefschrift eindigt met een Epiloog waarin een aantal problemen met betrekking tot NIRS aan de orde komen die in de (nabije) toekomst zeker opgelost dienen te worden. Afsluitend wordt de vraag "Is NIRS een speeltje of waardevol hulpmiddel?" positief beantwoord ten aanzien van de laatste keus.

Dankwoord

Nu het wetenschappelijke deel er op zit rest mij niets anders dan een ieder die aan dit onderzoek heeft meegewerkt of anderszins belangrijk was bij de totstandkoming van dit proefschrift te bedanken. Een aantal van hen wil ik op deze plaats in het bijzonder noemen.

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Curriculum Vitae

Willy Colier werd op 25 Juni 1960 geboren in Maastricht. In 1977 behaalde hij het HAVO diploma aan de K.S.G. Oud Vroenhoven te Maastricht. In datzelfde jaar begon hij een studie Fysische Technologie aan de H.T.S. te Heerlen. In het kader van deze studie deed hij onderzoek naar de toepassing van holografie om spanningsverschijnselen in materialen zichtbaar te maken. Van 1981 tot 1990 volgde hij een studie Technische Natuurkunde oude stijl aan de Universiteit Twente. In het kader van deze studie verrichte hij onderzoek naar de kwalificatie van apparatuur voor gebruik in een reactorgebouw van een kerncentrale bij Imatran Voima Oy te Helsinki, Finland. Het afstudeerproject betrof de realisatie van een confocale Ramanmicroscoop bij de vakgroep Biofysische Techniek onder leiding van Dr. Ir. Gerwin Puppels en Dr. Ir. Frits de Mul. Als student-assistent verzorgde hij gedurende een drietal jaren onderwijs. Hij behaalde het diploma Stralingshygiëne, niveau 3. Van 1990 tot 1995 was hij als Assistent in Opleiding werkzaam bij de vakgroep Fysiologie van de Katholieke Universiteit Nijmegen onder leiding van Prof. Dr. Berend Oeseburg. In 1992 werkte hij gedurende een drietal maanden aan de University of Keele, Department of Biomedical Engineering onder leiding van Prof. Dr. Peter Rolfe. In 1992 ontving hij de VSB-beurs voor veelbelovende onderzoekers. In 1994 ontving hij de Jonge Fysiologen prijs. Na een werkbezoek aan Prof. Dr. Marco Ferrari (Universita' Degli Studi Dell'Aquila, Italië) zal hij starten als wetenschappelijk onderzoeker aan de afdeling Neurologie van het Radboud Ziekenhuis in Nijmegen.

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Stellingen behorende bij proefschrift

Near Infrared Spectroscopy: Toy or Tool?

door

Willy N.J.M. Colier

1. Het weergeven van NIRS concentratie variabelen in arbitraire eenheden, zoals Vander's, is laakbaar.

2. De beschrijving in de leerboeken van vasovagale syncope als een plotsklaps fenomeen dient herzien te worden.

3. Pulse-oximetrie als patiënt bewakingssysteem geeft slechts informatie over de *aanvoer* van zuurstof.

4. Een reorganisatie kan een fantastische methode zijn om een illusie van vooruitgang te creëren en ondertussen demoralisatie, verwarring en ineffectiviteit voort te brengen.

   - Vrij naar Gaius Petronius, arbiter, A.D. 66-

5. De opkomst van de zwarte roodstaart in Nederland, wiens natuurlijke habitat een steenachtig gebied is, wijst op het veranderen van Nederland in een steenwoestenij.

6. Het is vaak minder belangrijk te weten welke ziekte iemand heeft dan te weten wie de ziekte heeft.

   - Oliver Sacks, neuroloog -